Laser Fabrication of Combinatorial Libraries for Polymer and Biotechnology Development

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Monday, April 26, 2004

NCMC-5: Combinatorial Processing & Characterization

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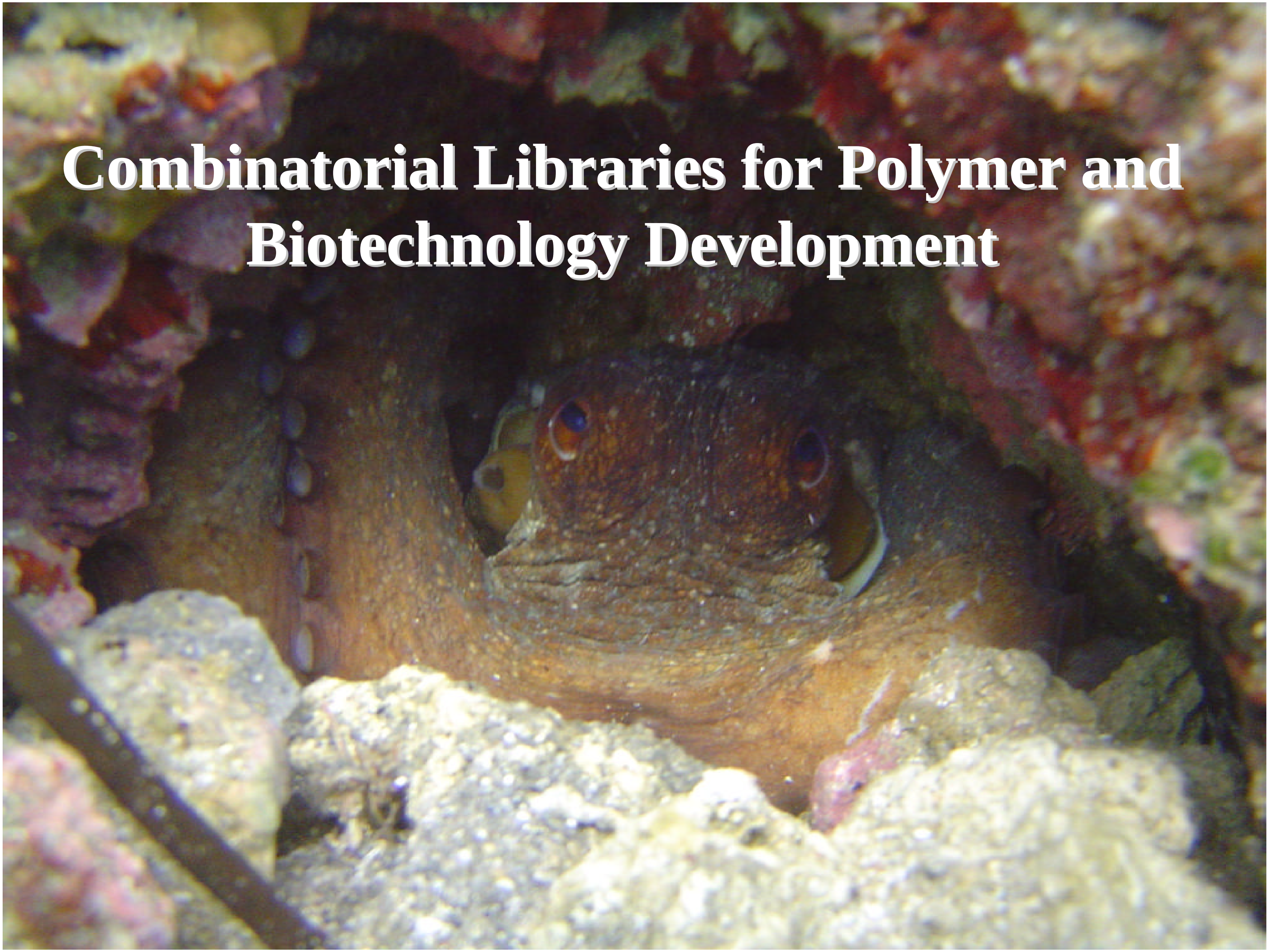
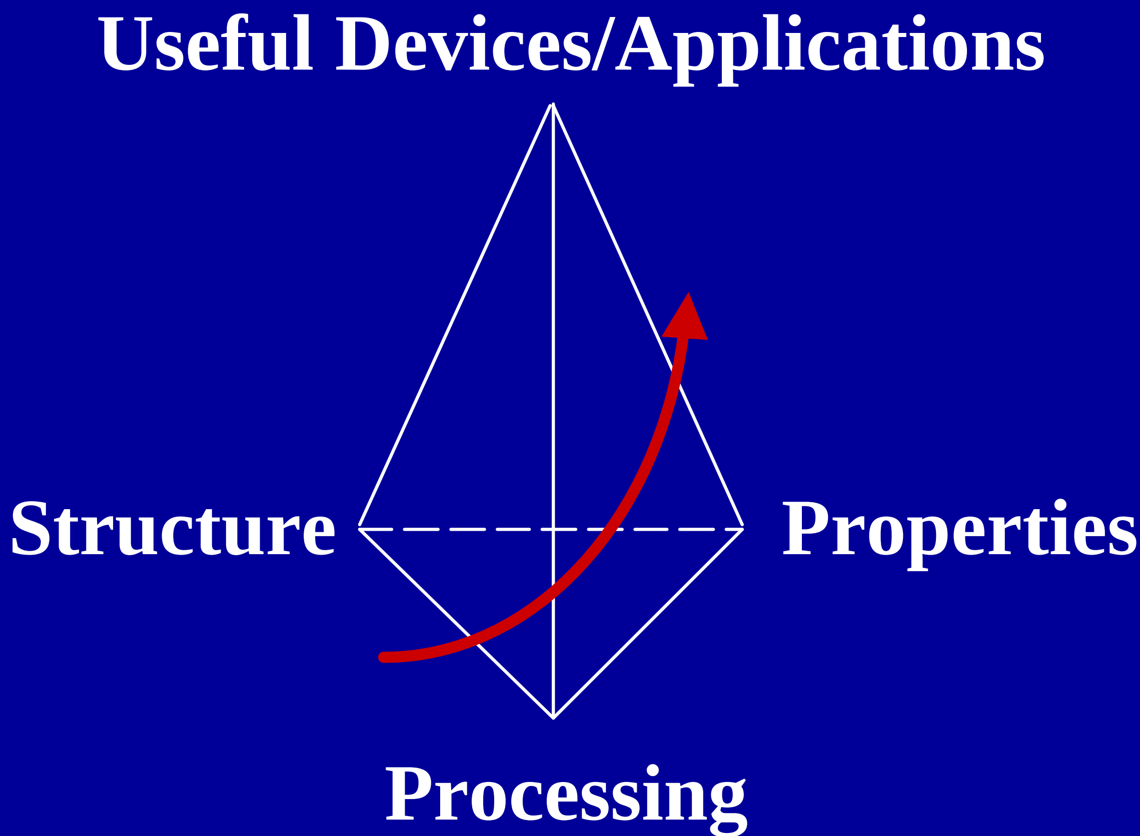
Outline

- Brief Introduction to Combinatorial Processing
- Introduction to MAPLE DW
  - Direct Writing Electronics
  - Direct Writing Biomaterials
  - Rapid Prototyping Biological Systems
- Application of MAPLE DW to the Fabrication of Combinatorial Libraries for Polymer and Biotechnology Development
- Conclusions


Combinatorial Libraries for Polymer and
Biotechnology Development

New Materials Needed for Next Generation Applications

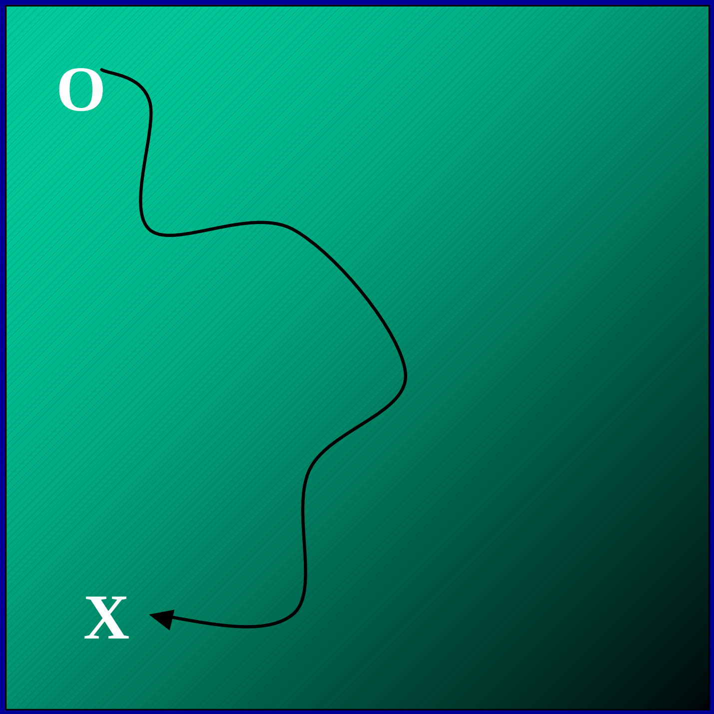


Improvements in Materials Processing Will Result in Improved Capabilities and New Devices

New Methods of Rapid Material Discovery are Needed

- Conventional Approach to Material Discovery Has Been Edison-ian (Slow and Serendipitous Trial and Error Study)

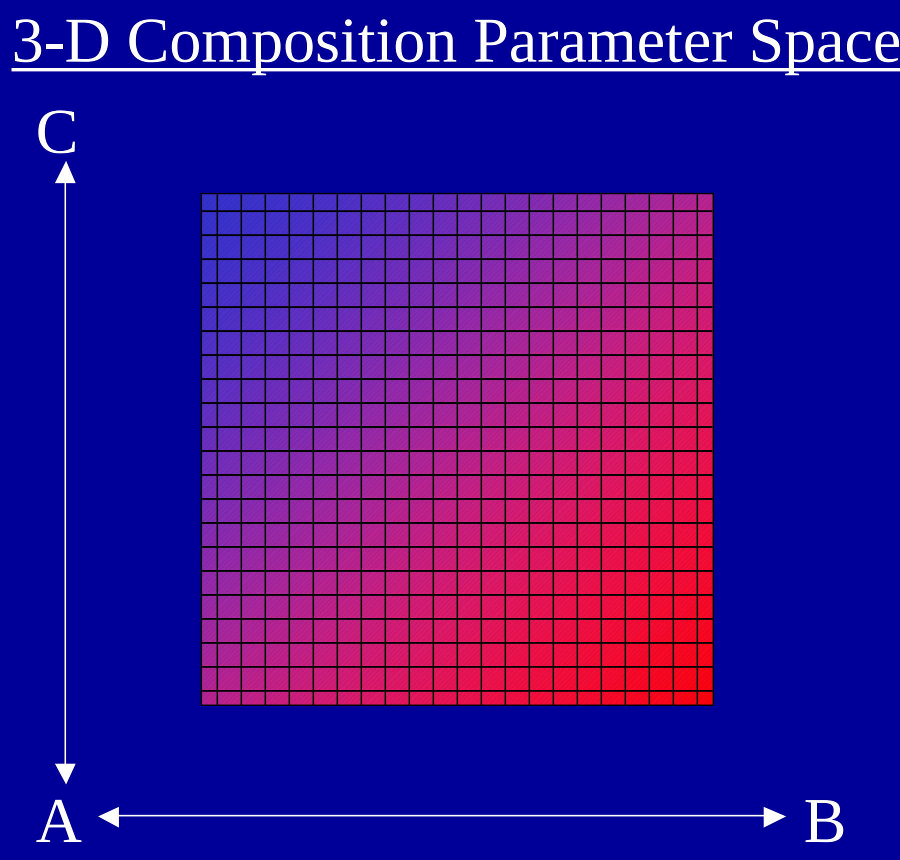
2-D Parameter Space



Combinatorial Synthesis

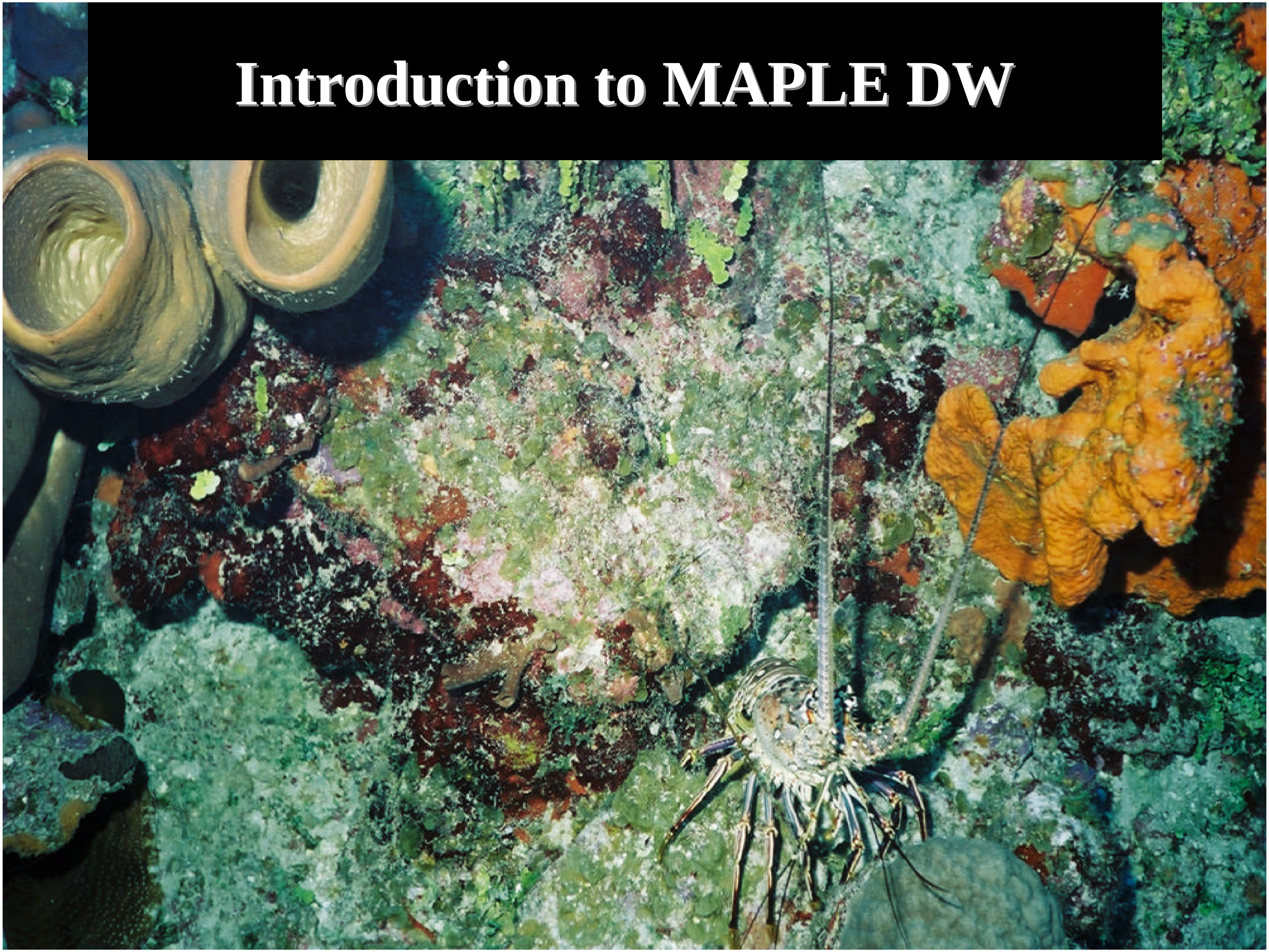
- Combinatorial Approaches Have Revolutionized Materials Discovery
  - Automated Parallel Synthesis and High Throughput Screening Systems
  - Very Large Numbers of Compositions Can Be Evaluated Simultaneously
  - Dramatically Reduce the Development Cycle — Speeding Commercialization
- Combinatorial Sample Library Is a 2-Dimensional Scan of Elemental Composition
- Environmentally Sound
- Combinatorial Experiments Conveys the Depth of Material Science In a Single Sample

Combinatorial Processing of Polymer and Biomaterial Libraries

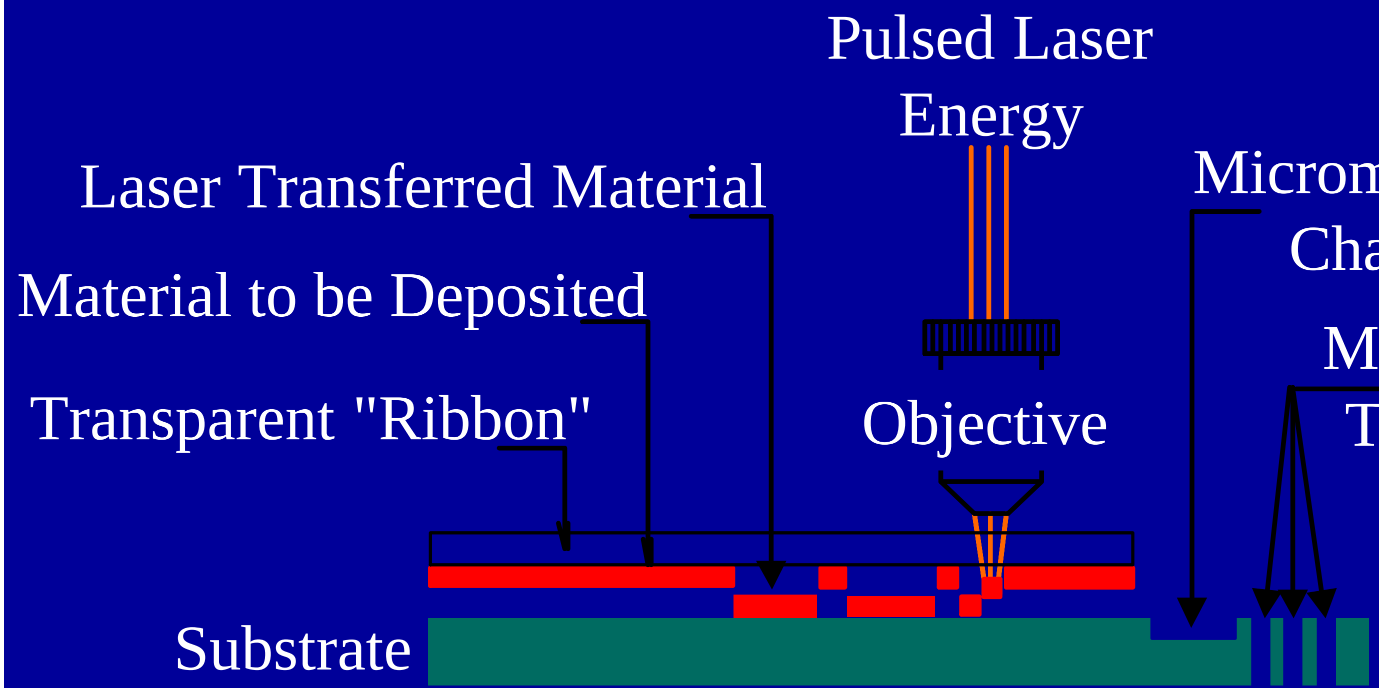


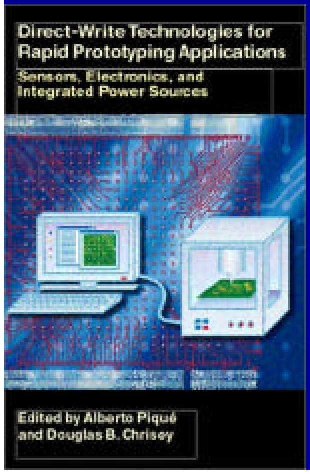
Small Amounts of Biomaterials That Are Densely Packed That Can Be Individually Analyzed!

Introduction to MAPLE DW

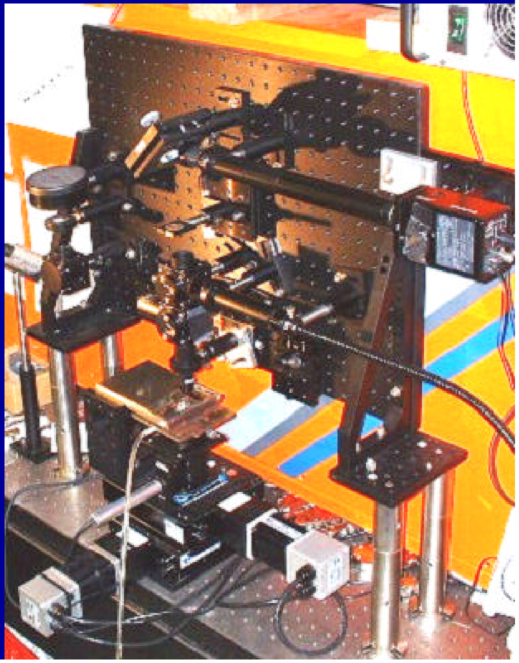


Matrix Assisted Pulsed Laser Evaporation Direct Write (MAPLE DW)



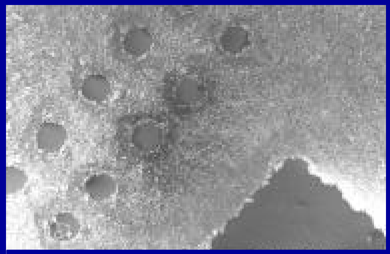


Done Under Ambient Conditions!



“Ribbons” in MAPLE DW

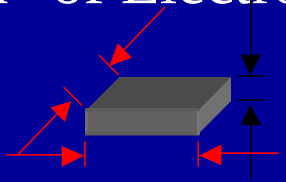
The Interaction of the Laser with the Ribbon is the Novelty in MAPLE DW. It is Both a Liability and an Asset.



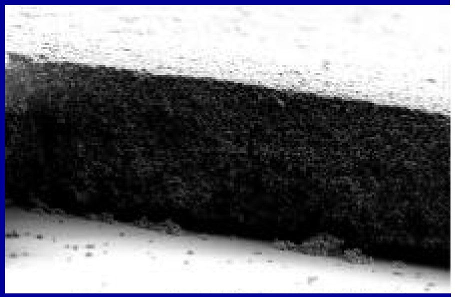
Ribbon

Liability: Ribbons are Difficult to Fabricate.

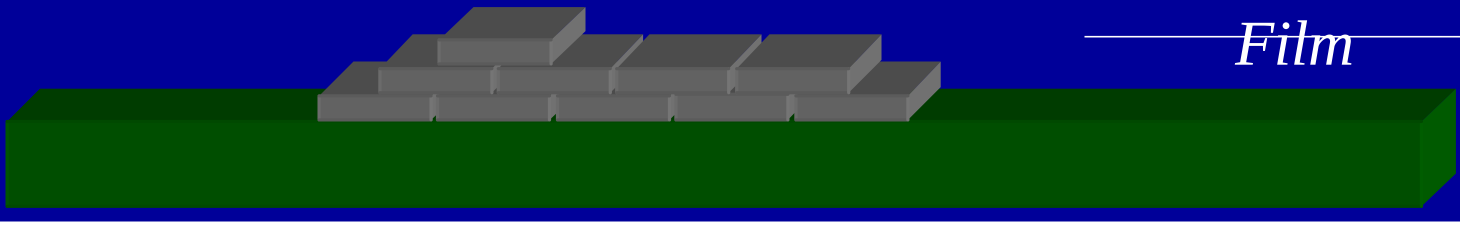
Asset: Ribbons Effectively “Quantize” the Material Transferred Making MAPLE DW Coatings Highly Reproducible. Each Laser Pulse Deposits an Identical Mesoscopic “Brick” of Electronic Material.



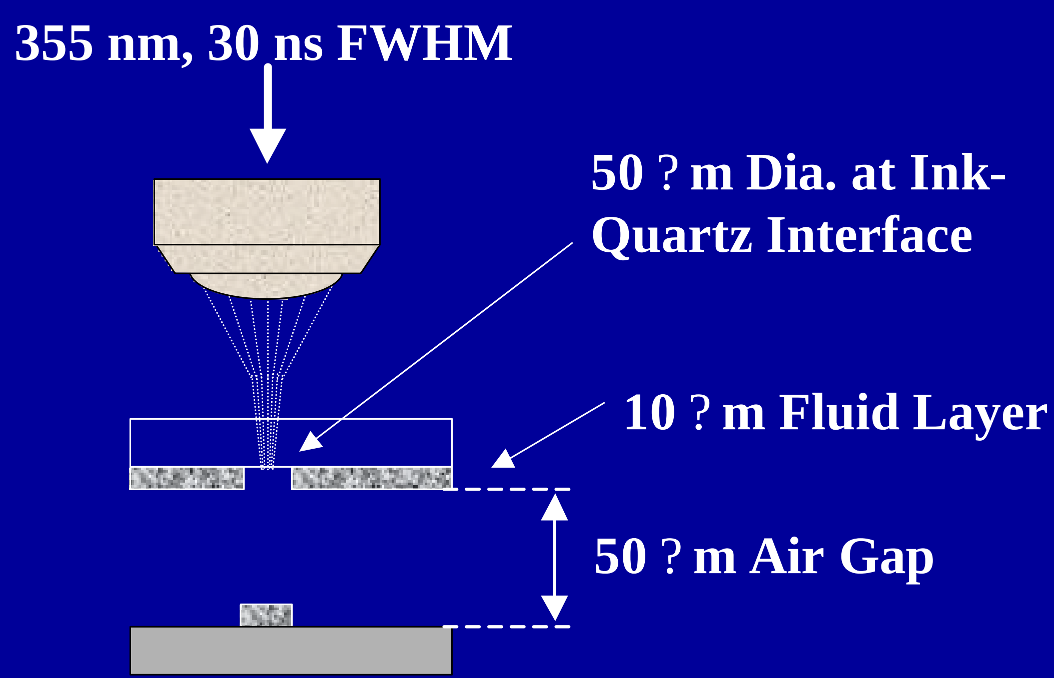
(Beam Cross-Section X Ribbon Thickness)

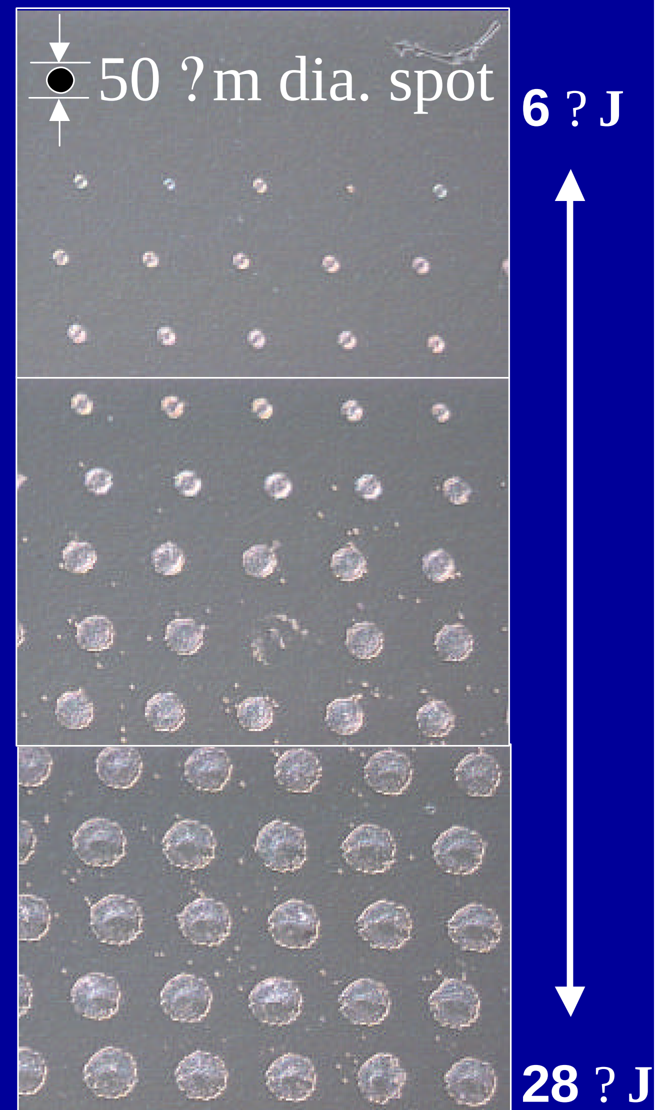


Film



Transferred Spot Size vs. Fluence

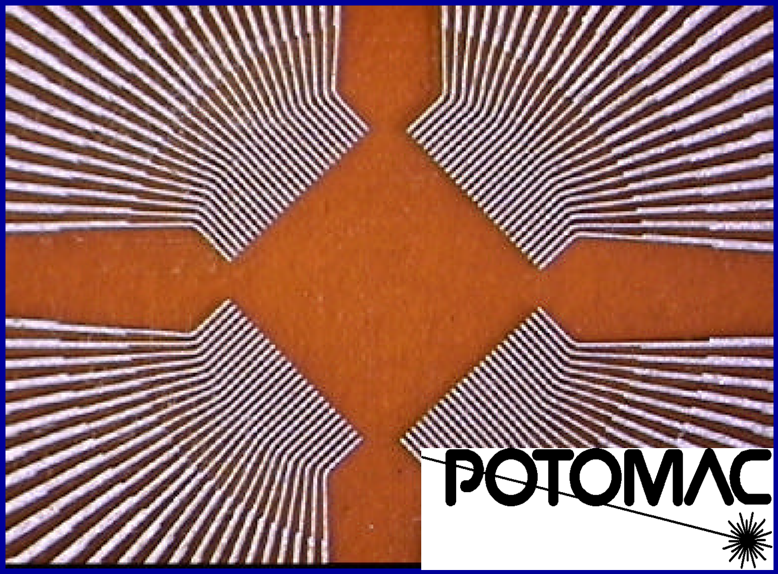




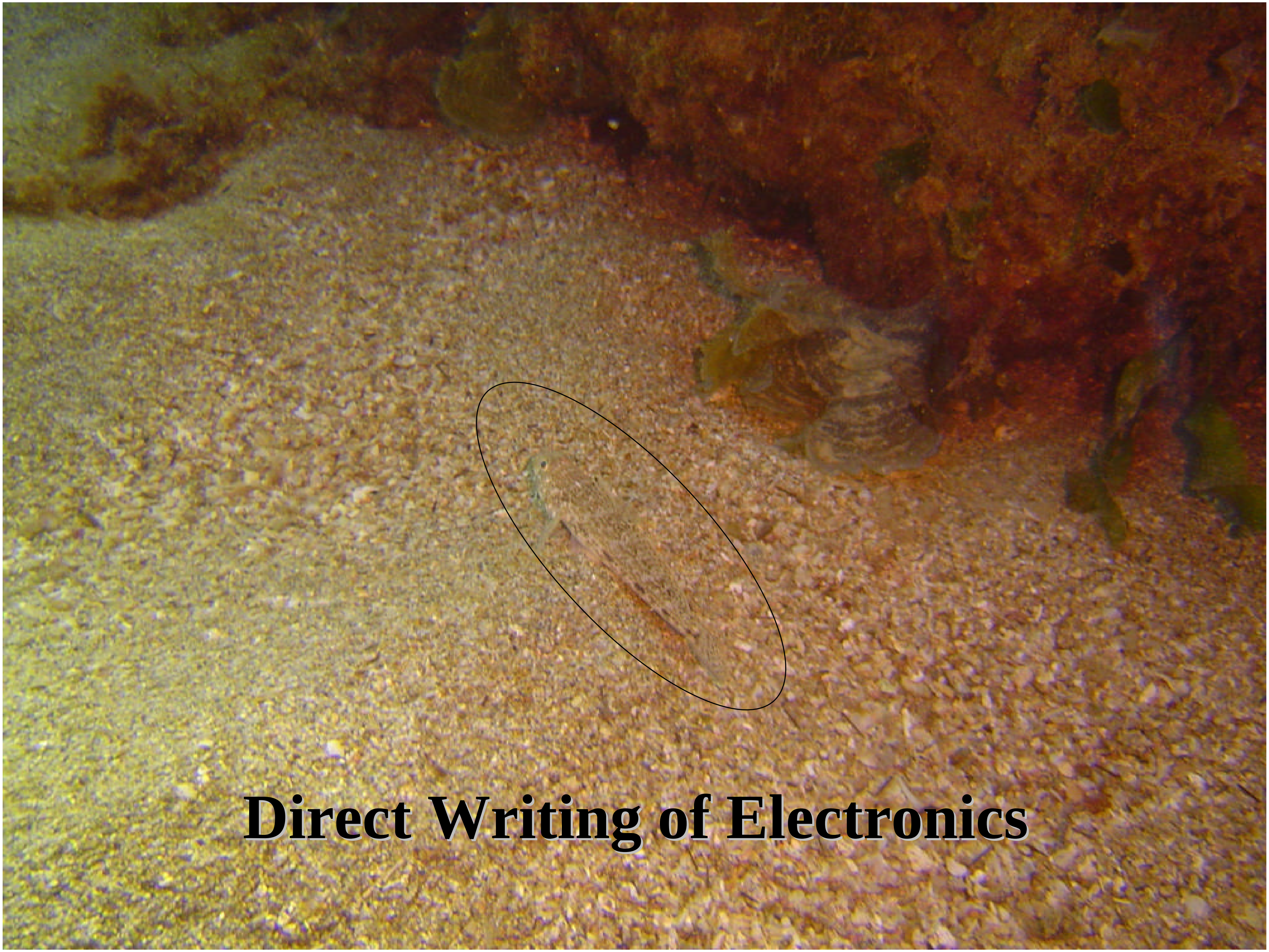
Solute: BaTiO$_3$ Nanopowder, 150nm dia.
Matrix: ? -Terpineol + Rheological Modifiers

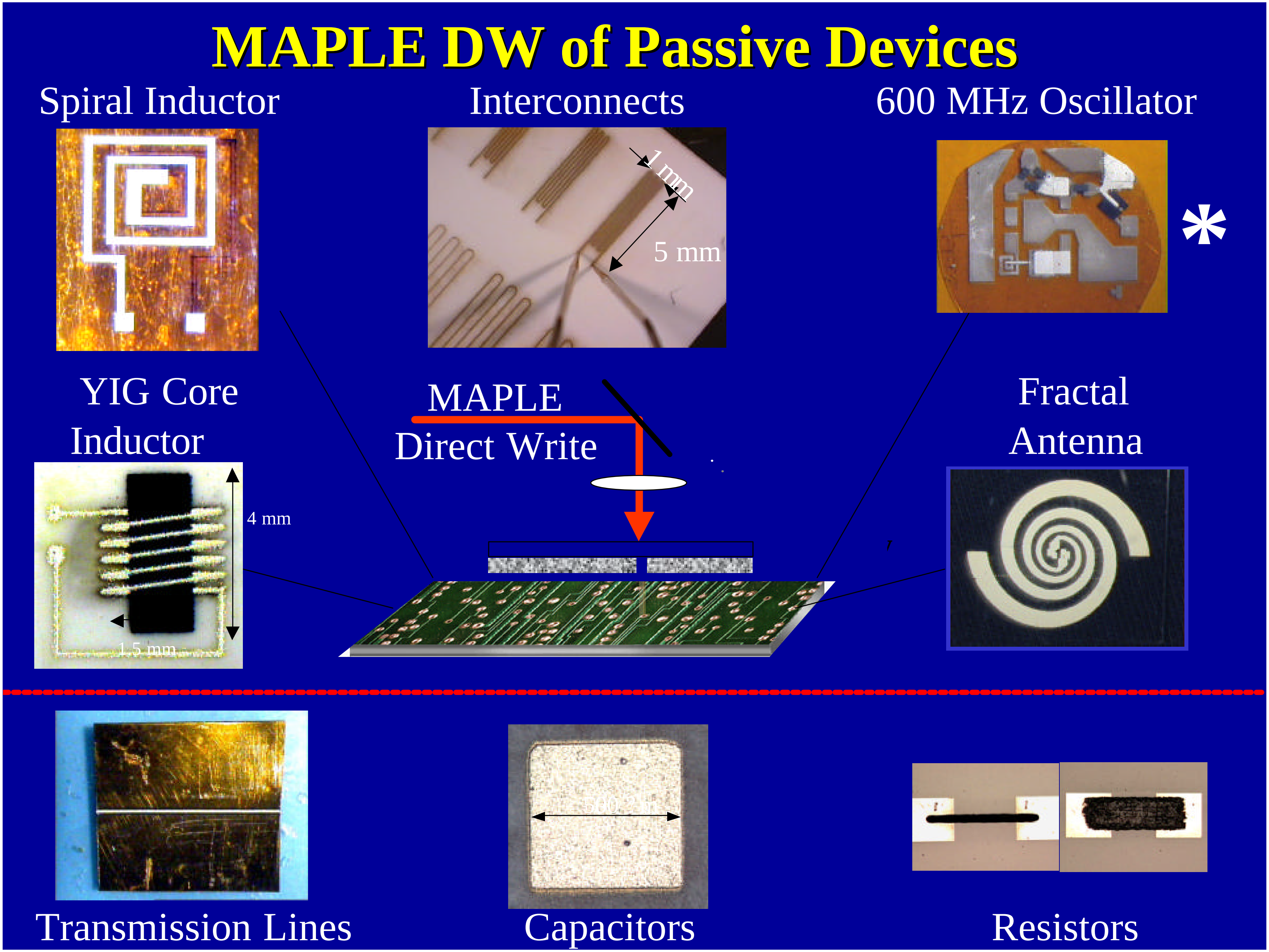
Transferred Spots Smaller Than "Spot Size" Just-Above-Threshold

Fan Out Network for Contact Redistribution

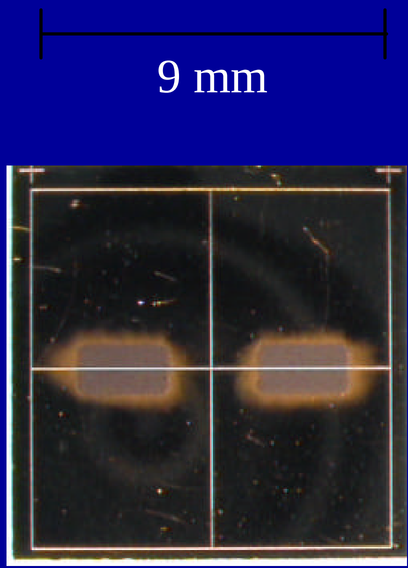


- MAPLE DW Is a Serial Process, But With High Rep. Rate Lasers (100 kHz), Small Lot Size Manufacturing is Possible
- Can Fabricate Electronics, Power, and Communication and I/O Devices As Well As Viable Cell and Biomaterial Patterns


Direct Writing of Electronics

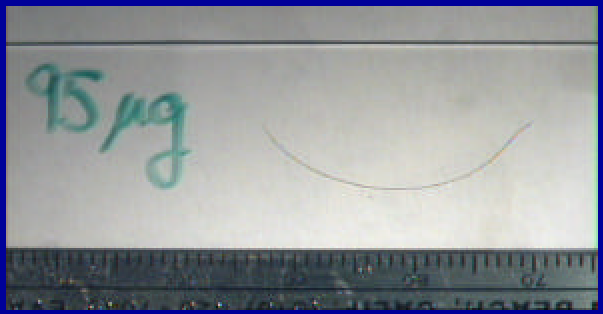

MAPLE DW of Passive Devices
Spiral Inductor
Interconnects
1 mm
5 mm
600 MHz Oscillator
*
YIG Core Inductor
4 mm
1.5 mm
MAPLE Direct Write
Fractal Antenna
Transmission Lines
Capacitors
Resistors

Laser Direct-Write and Micromachining of Planar Hydrous RuO_2 Ultra-Capacitor Cells



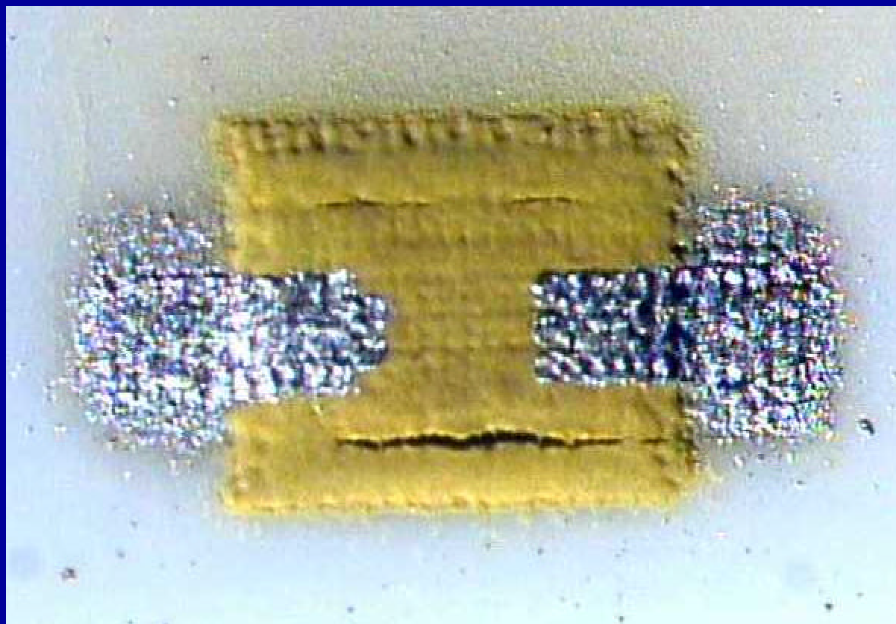
Actual Machined Ultracapacitor Cells

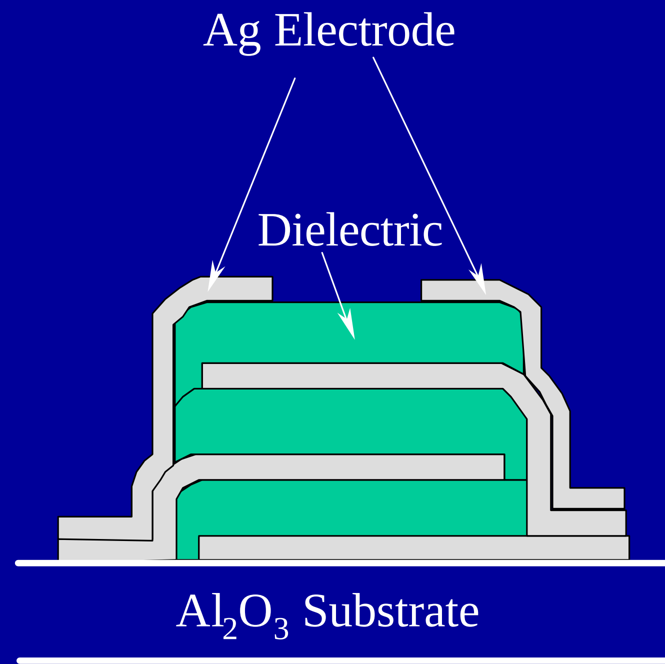
Volume ~ 2 x 10^{-5} ml
Mass < 0.1 mg/cap
The Weight of a Human Hair

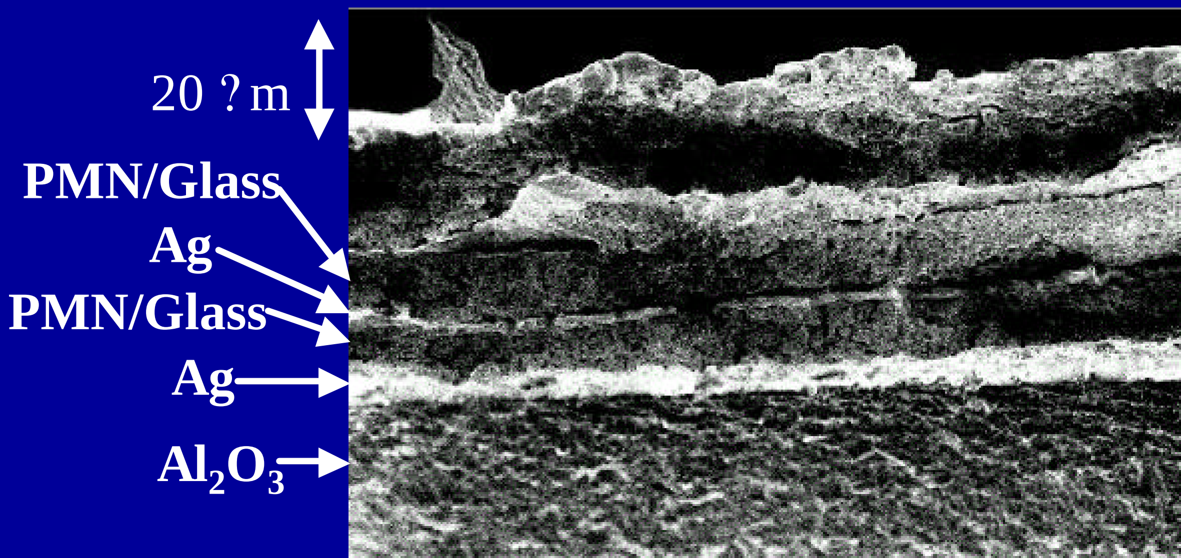


Our Technique is Unique Since it Can Direct-Write "Inks" Containing the Active Material (RuO_x) + The Electrolyte (H_2SO_4)

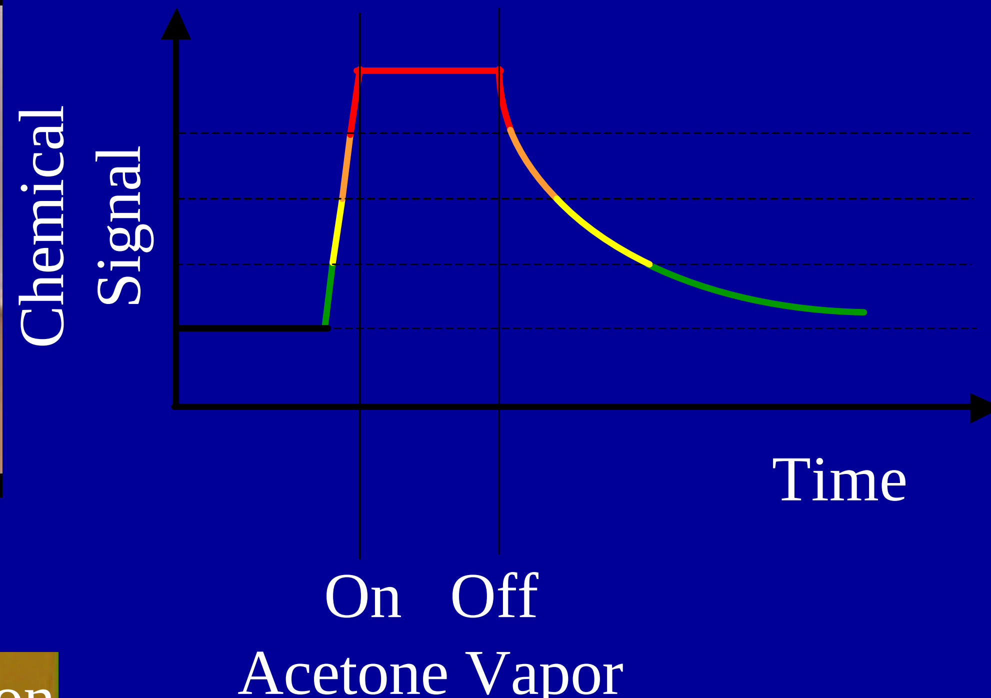
Multilayer Capacitors

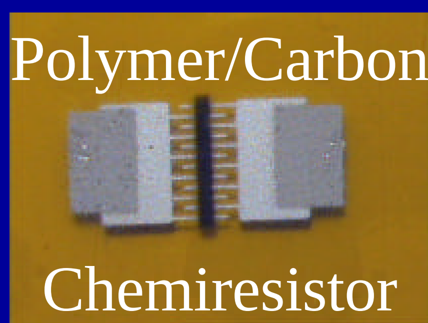






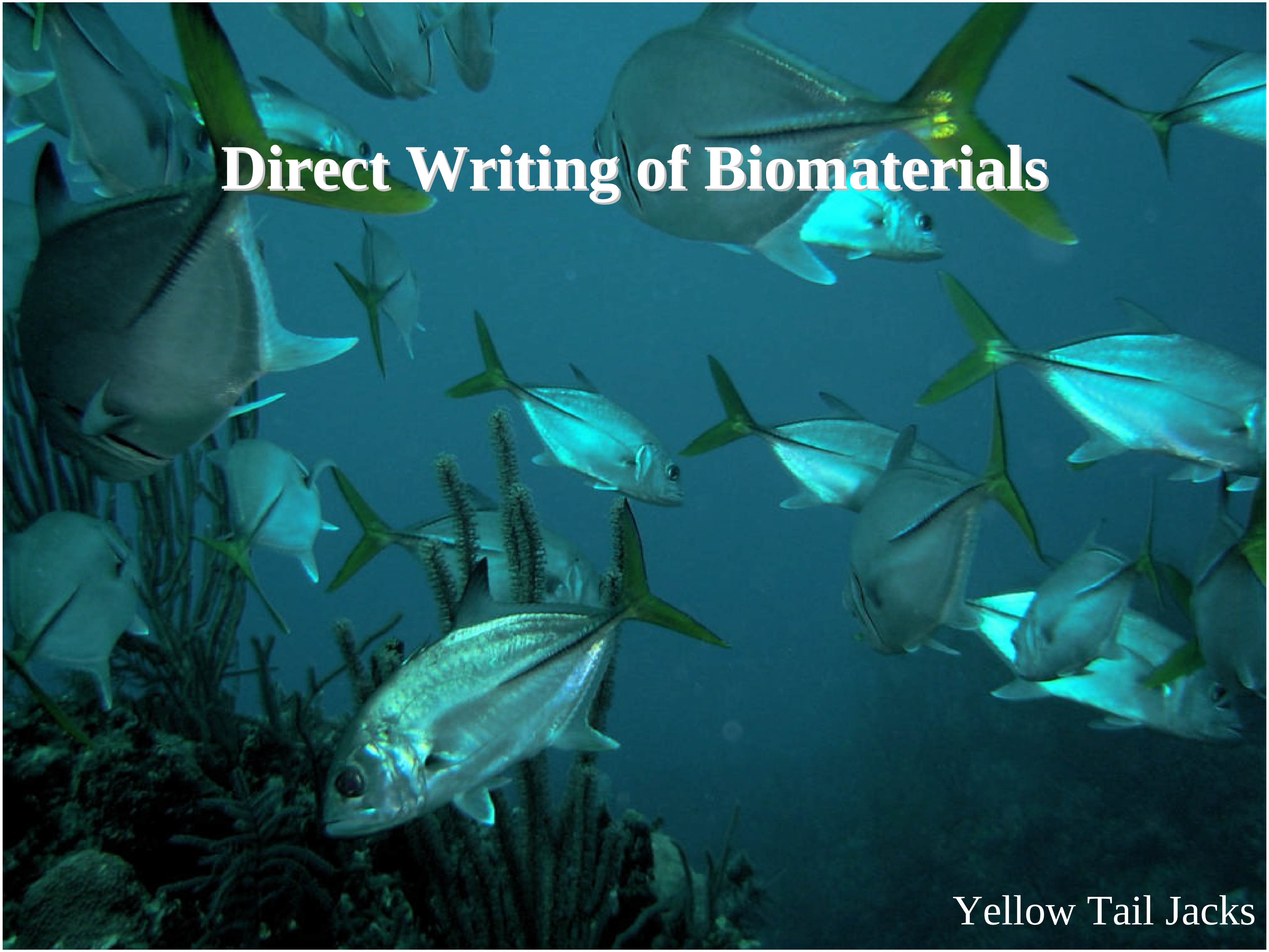
Prototype Chemiresistive Sensor Subsystem





Chemical Signal is Rapidly Adsorbed to Chemoselective Polymer (Detected as Resistance Change) And More Slowly Desorbed!

Direct Writing of Biomaterials



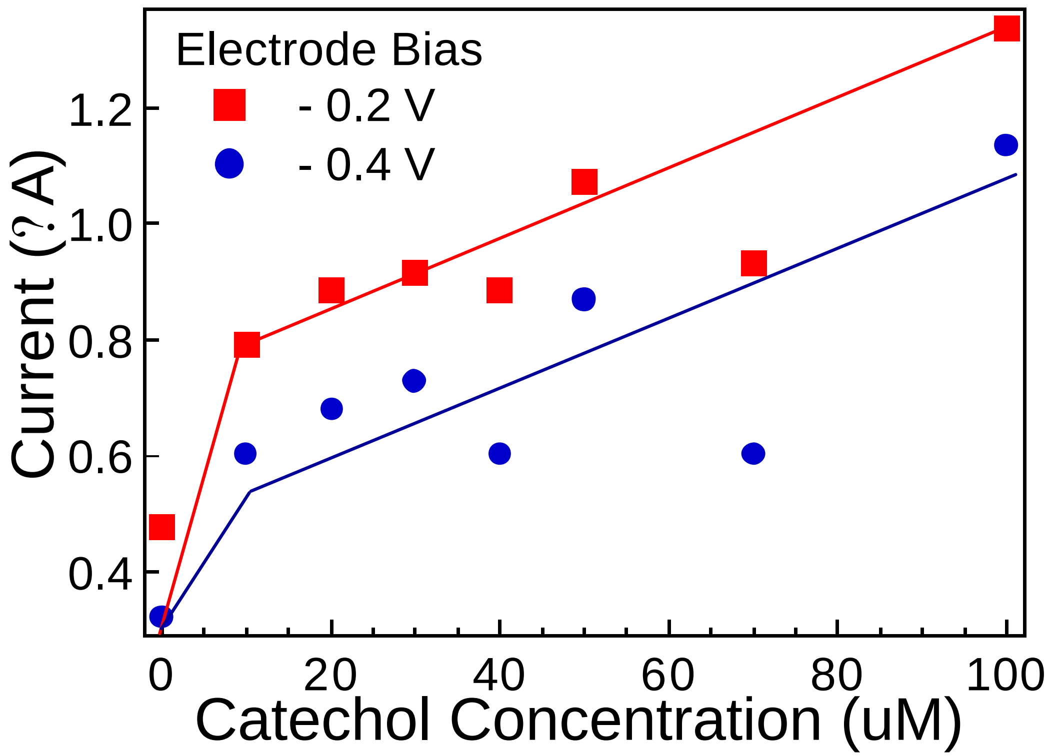
Yellow Tail Jacks

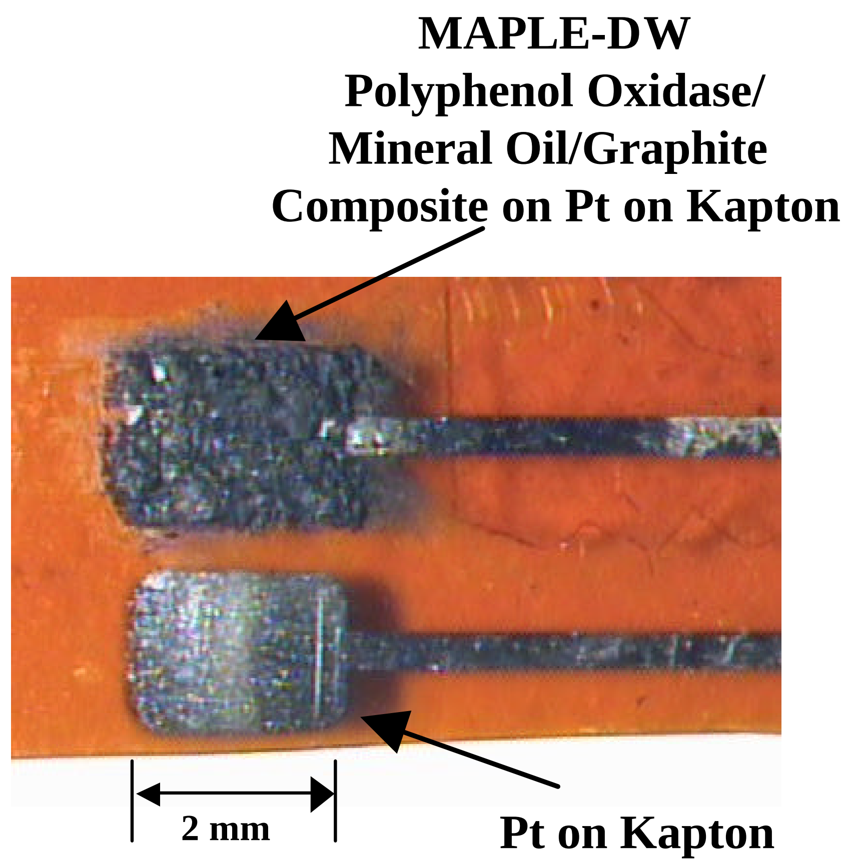
Diagnostic Sensitivity Requirements

Agent	Infective Dose	Agent	Infective Dose
Anthrax	8,000 to 50,000 spores	Smallpox	10-100 organisms*
Brucellosis	10-100 organisms	VEE	10-100 organisms
Plague	100-500 organisms	Viral Hemorrhagic Fevers	1-10 organisms
Q-fever	1-10 organisms	Botulinum Toxins	~70 ng
Tularemia	10-50 organisms	Staph Enterotoxin B	~30 ng

Biomaterial Sensors Needed for Biological Warfare Agents!

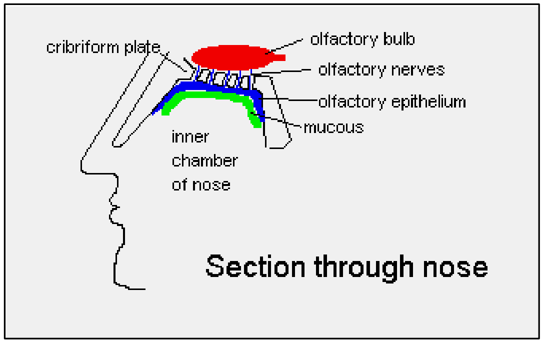
Fabricating a Miniature Dopamine Biosensor Using MAPLE DW



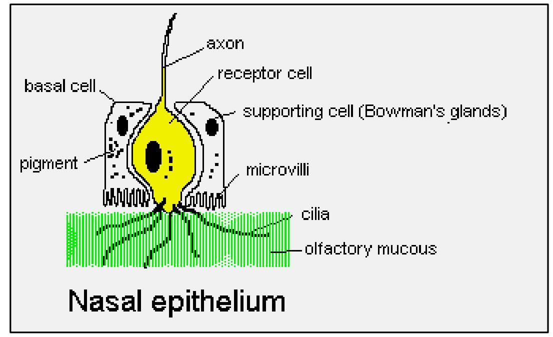


Current Increases With Analyte Concentration

Utilize Engineered Tissue Constructs With Transducers For A Bioelectronic Nose

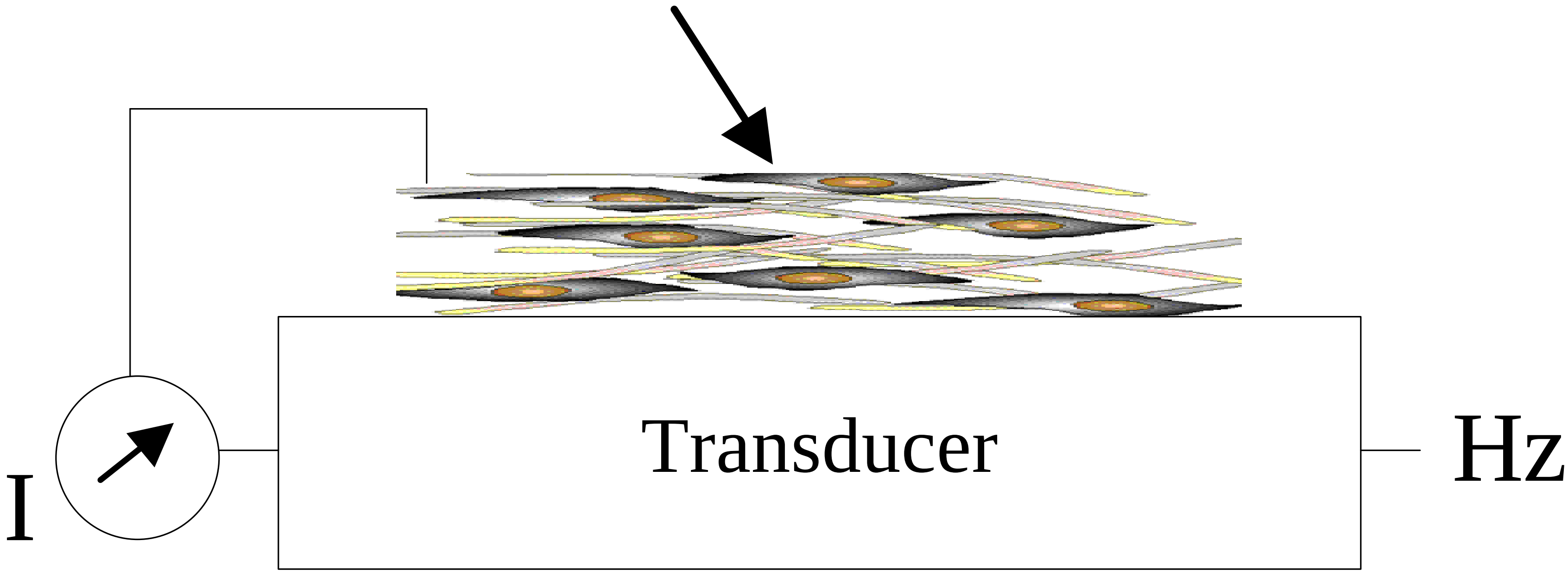


Section through nose



Nasal epithelium

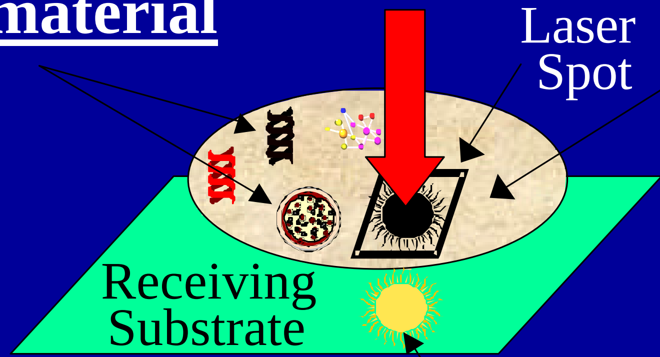
Engineered Tissue Construct: Olfactory Epithelium



MAPLE DW Approach to Laser Transferring Living Cells and Biomolecules

Transfer Biomaterial

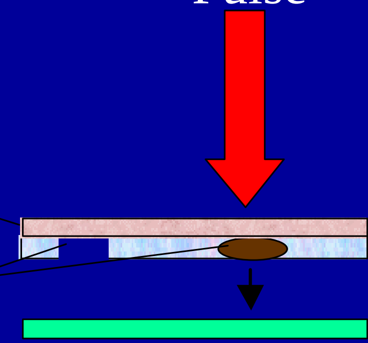
- Proteins
- Antibodies
- Enzymes
- Antibiotics
- DNA
- RNA
- Living Prokaryotic and Eukaryotic Cells



Matrix Material

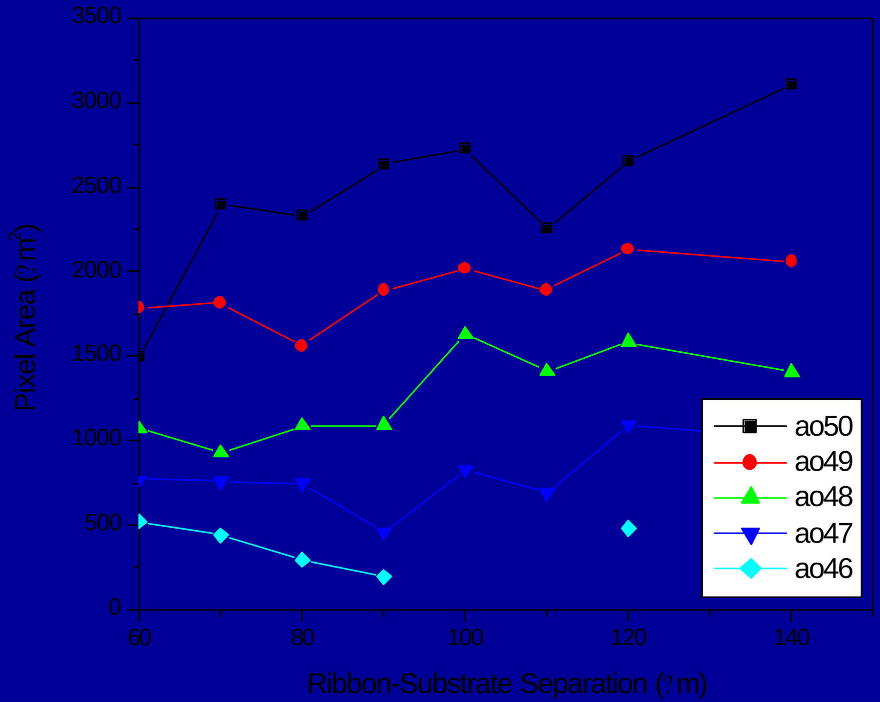
- Polymers
- Hydrogels
- Sol-Gels
- Proteins
- Aqueous Solution

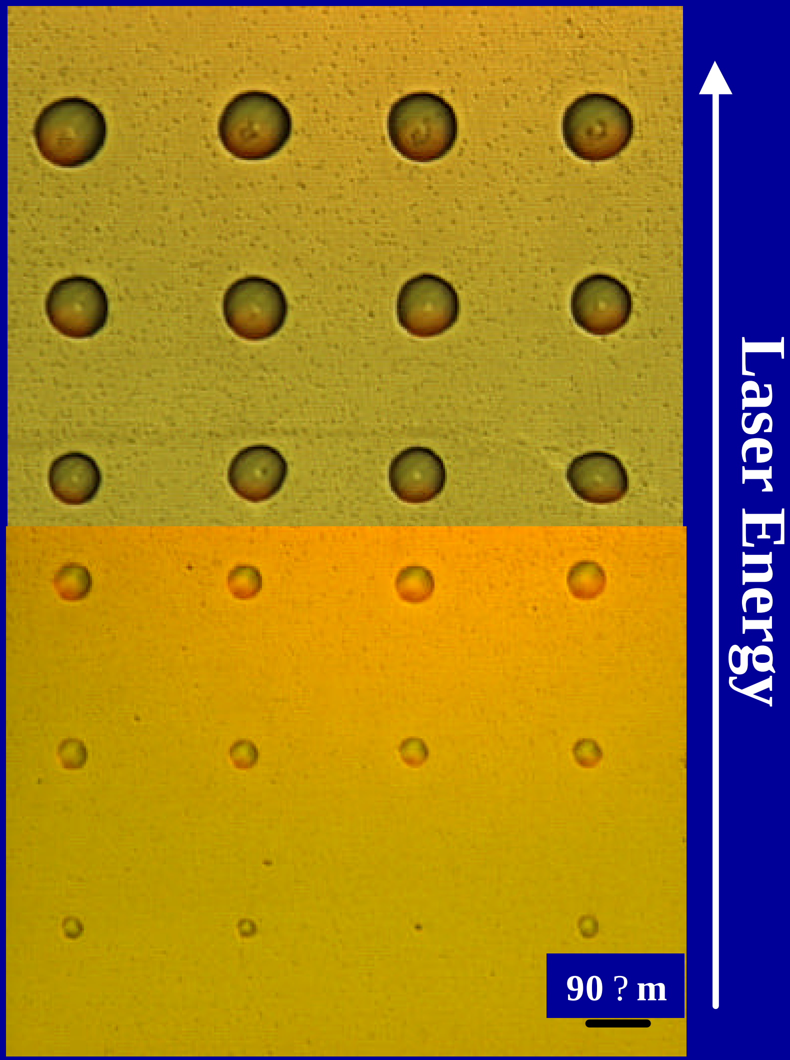
Side View



Assemble Raw Biomaterial Components ? Living Biological Systems

Picoliter-Scale Dispensing of Biological Solutions

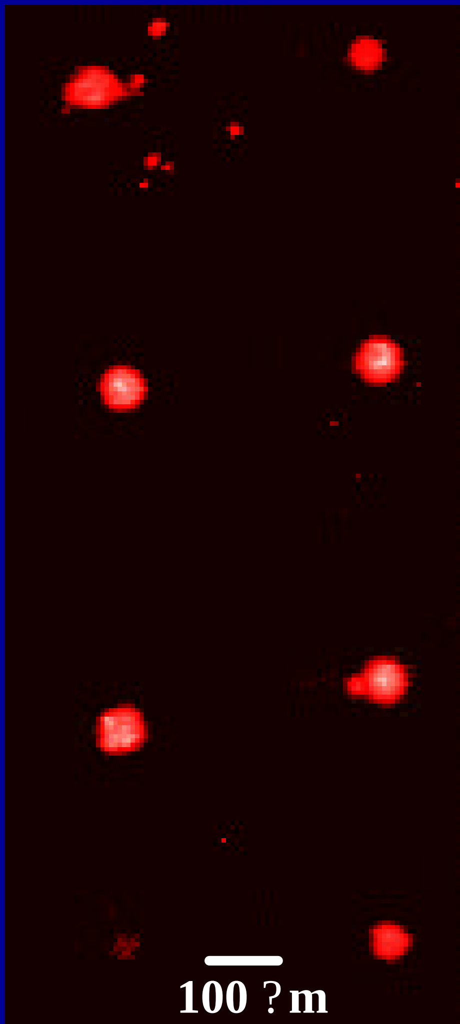




Laser Spot Size = 125 ? m

- *Preserves Spot Resolution over Large Transfer Distances (Microwells, Microchannels, etc.)*
- *Demonstrated Volume Transfers From 10 fL to 10 nL/shot*

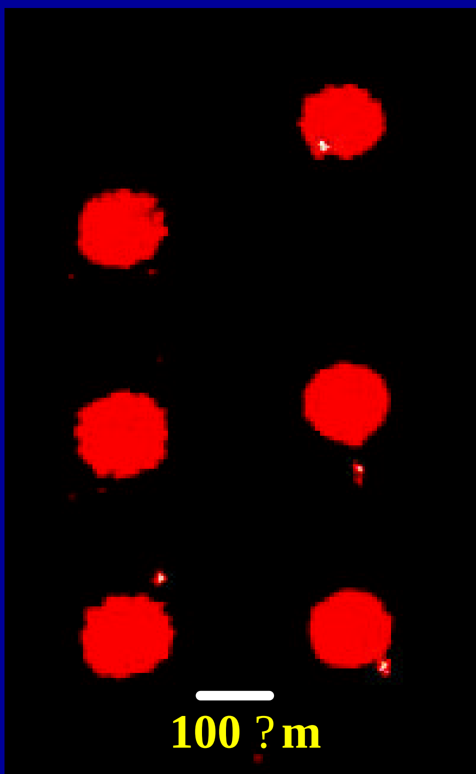
Protein Microarray Fabrication Using MAPLE DW



635 nm Fluorescence Intensity

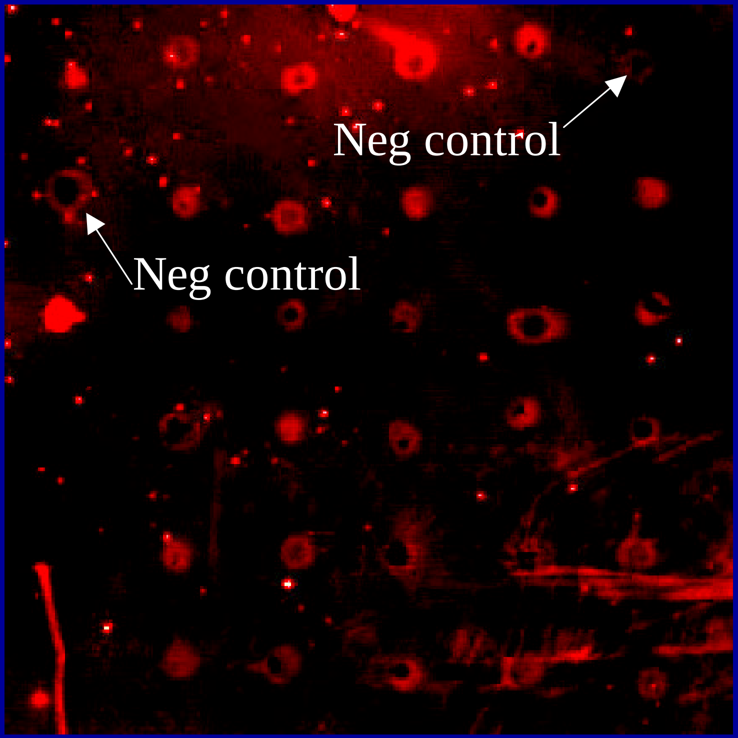
- Biotinylated BSA Microarray
- Substrate: Nitrocellulose-Coated Glass Slide
- Spot Size: 50 ?m
- Protein Volume Dispensed per Spot: 100 pL
- Fluorescent Tagging Performed with Cy5-Labeled Streptavidin

Single Element Antibody Microarray



- Anti-BSA Microarray
- Substrate: Nitrocellulose-Coated Glass Slide
- Spot Size ~ 150 ?m
- Antibody Volume/Shot ~ 1 nL
- *BSA Successfully Identified out of a Mixed Protein Solution (~10* ?g/mL)

30 Different Antibodies Specific to Cell Signaling Pathway Proteins

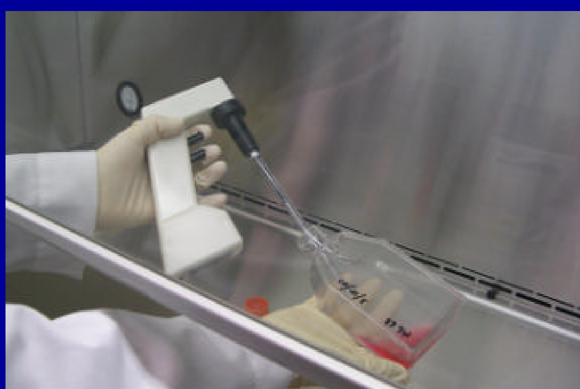


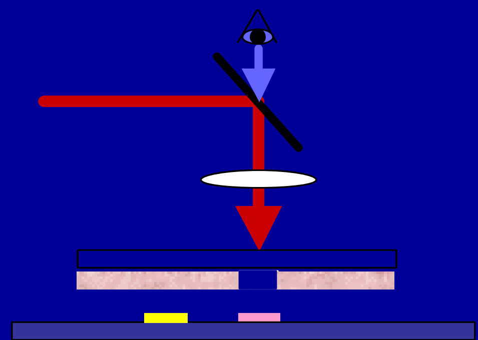
- ~ 1 nl Antibody Per Spot
- Protein Solution Came From Cells With All Signaling Pathways "Turned-on" So We Should Essentially See All Spots Light up

MAPLE DW of Active Proteins

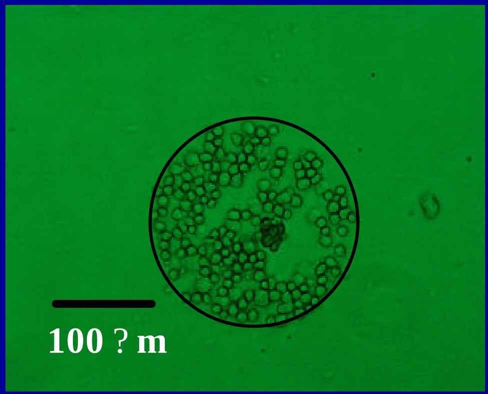
- Advantages Over Conventional Techniques
  - Works With ANY Starting Material (Viscosity, Powders, Varying Conditions
  - Reproducibility
  - CAD/CAM
  - Reduced Spot Size <30 ?m
  - Increase Material Utilization/Efficiency by 10^5
  - Works on Planar Substrates and Microwells
  - One System Does Complete Array of Multiple Array Elements

Cell Transfer by MAPLE-DW





Transfer Movie

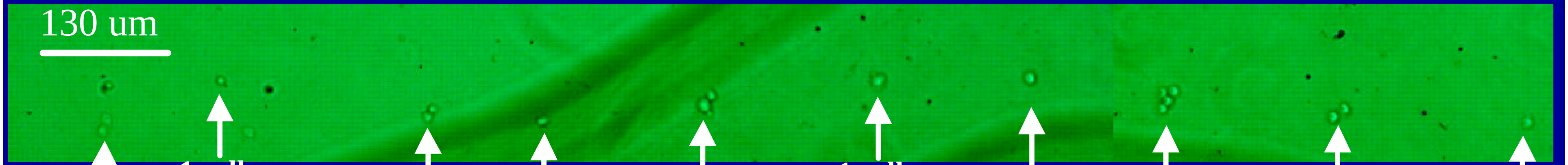


Preparation — Transfer — Analysis →

Culture — < 1 msec — *Culture*

Single-Cell Resolution and Deposition

Line of Single Human Osteoblasts by Laser Transfer



Do We Genetically Alter the Cells?

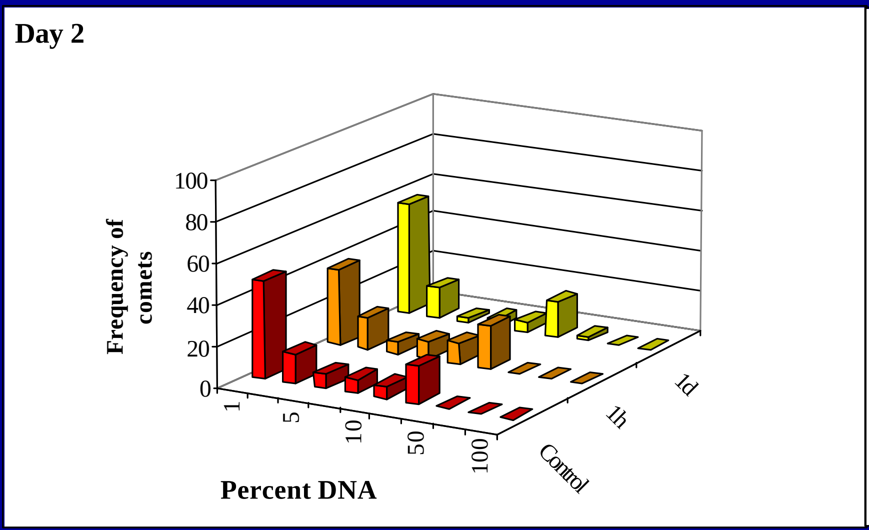
- We Use an Arf Excimer Laser at 193 nm = ? ?(UV) That Is Known to Damage DNA When Directly Exposed to Mammalian Cells.

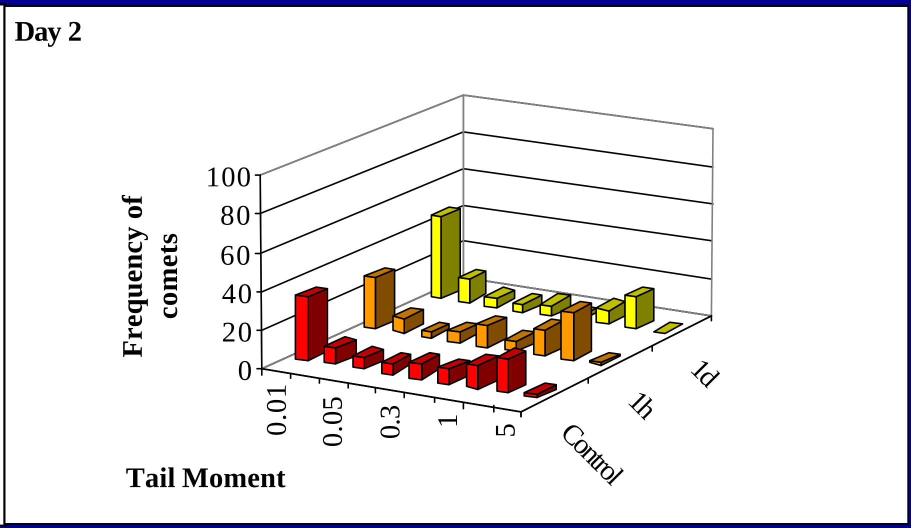
- Therefore, It Is Possible That Diffuse Laser Light During Our Processing Could Induce Single or Double Strand Break in the Cell's DNA

- We Performed a Neutral Comet Assay on a Group of Transferred P19 Cells and Found No Evidence of Double Strand Breaks in Those Cells

- Alkaline Comet Assay, Which Is More Sensitive to Dna Damage, Also Showed No Damage

Comet Test Results on Laser-Transferred P19 Cells

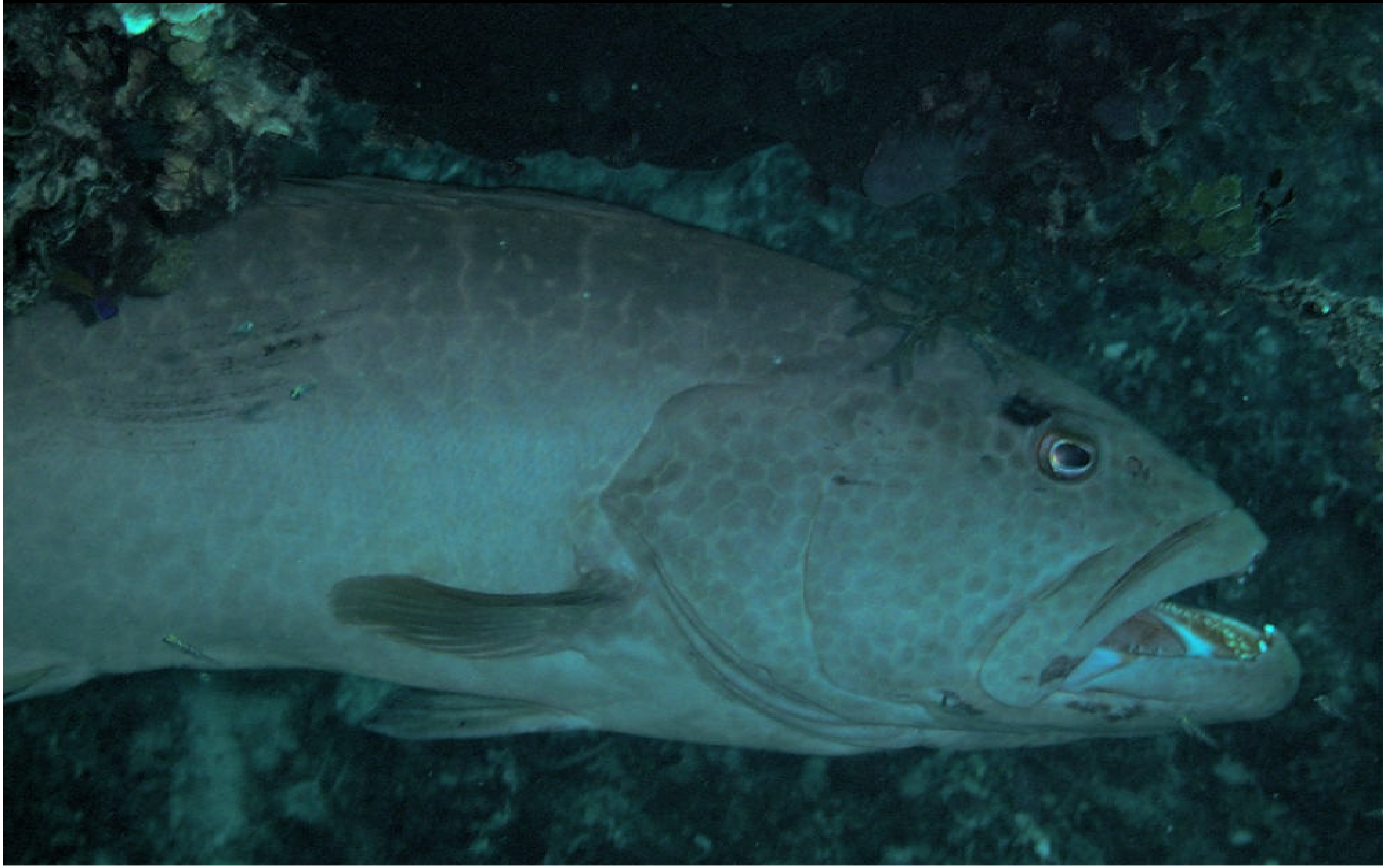




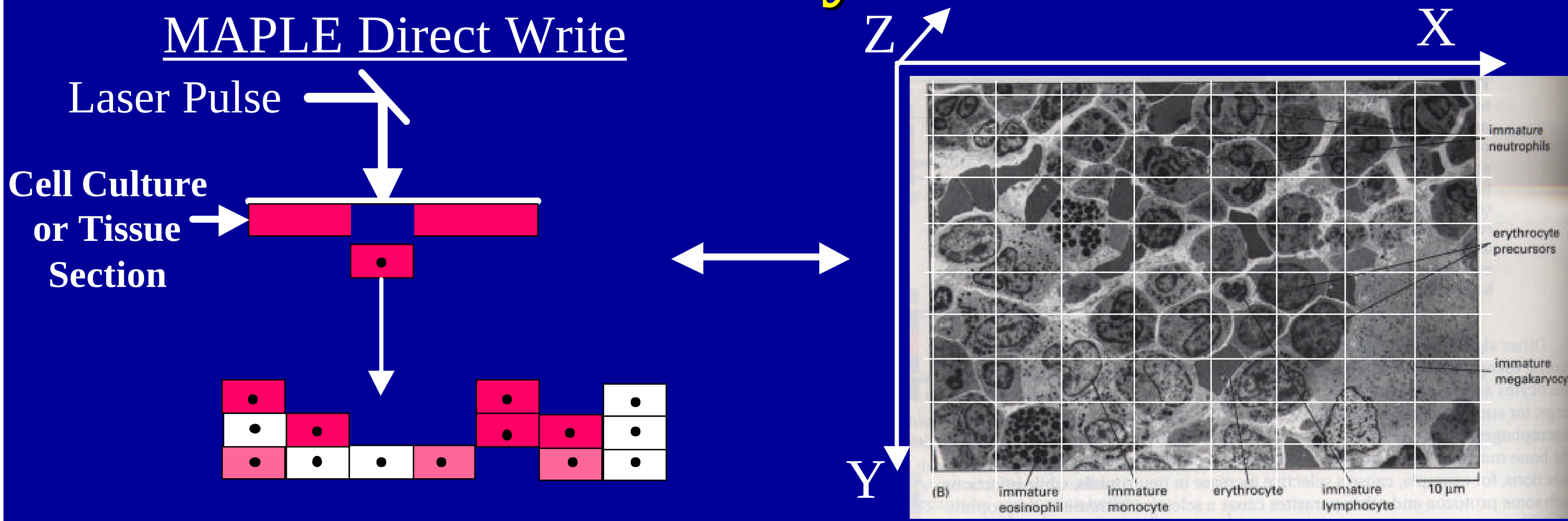
- Distributions for 1 hr and 1 Day Post-Transfer Are Nearly Identical To Control Within Statistical Noise

***Minimal to No* Double Strand Breaks in Laser-Transferred Cells**

Rapid Prototyping Living Biological Systems



What if Tissue Could Be Constructed Cell-by-Cell?



- ***Is MAPLE DW Suited For Cell-by-Cell Tissue “Construction”?***

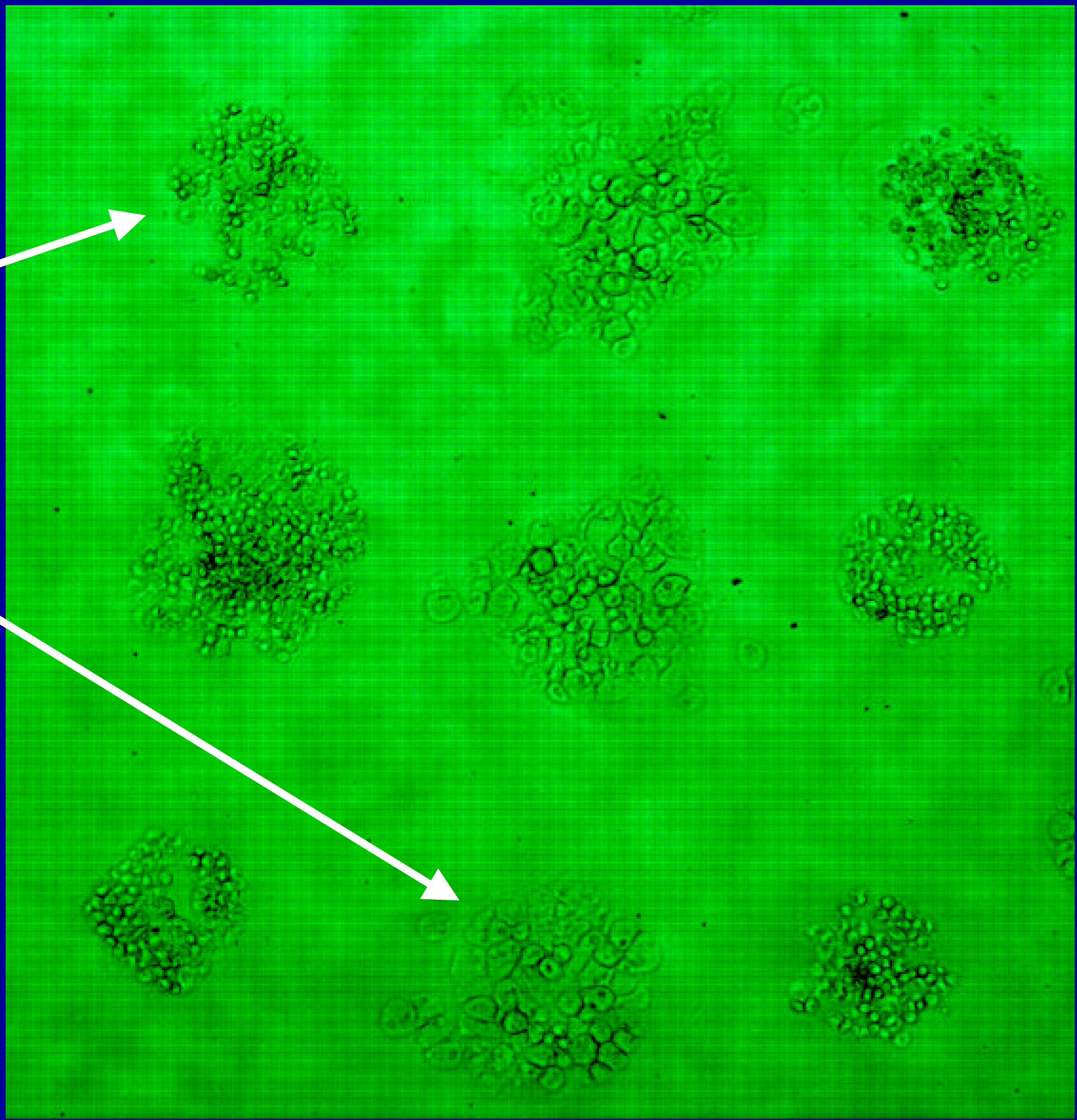
-Rapid, Computer-Controlled Placement of Different Materials at 10 to 100 ?m
-Multiple Cell Types
-Molecules Like Growth Factors, Recruitment Factors, Differentiation Chemicals
-Novel Scaffolding Materials (Inorganic/Organic Composites)
-Vascularization (Constructs >1 mm)

Two Cell Type Microarray

Osteoblasts

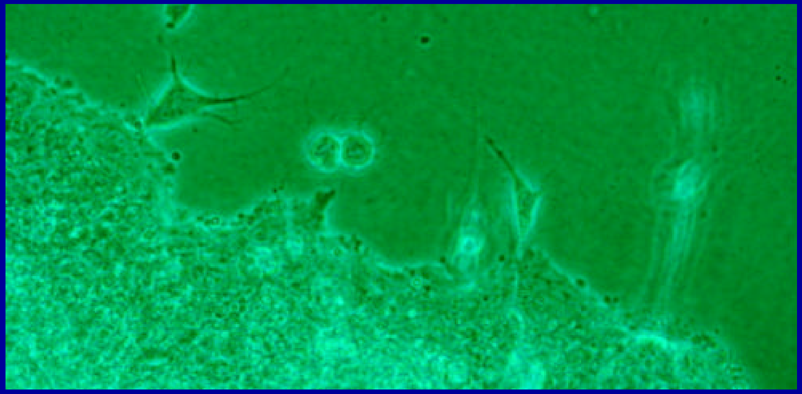
Cardiocytes

Note the Difference
In Cell Morphology



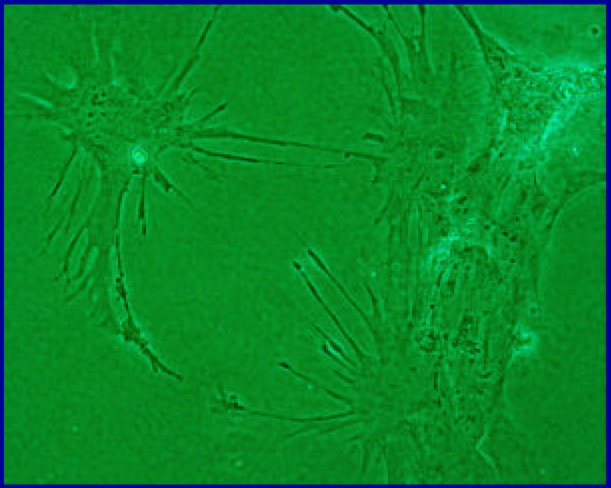
Mouse Pluripotent Cell Differentiation Post-Laser Transfer

Using DMSO



Muscle Fiber Growth

Using RETINOIC ACID

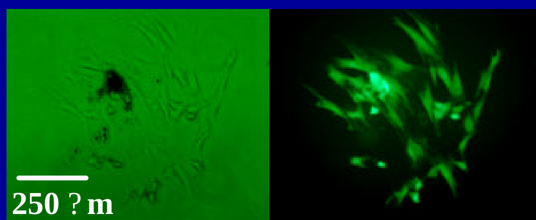


Observed Dendrite Growth

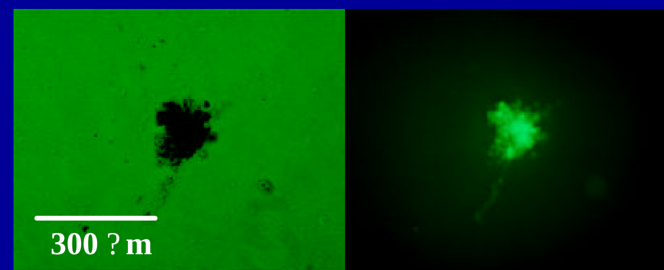
Transferred Pluripotent Cells Differentiate!

Next Step to Demonstrate Differentiation Using Surface Antigen and Cytoskeleton Markers

Single Shots and Multiple Shots of Rat Cardiac Cells



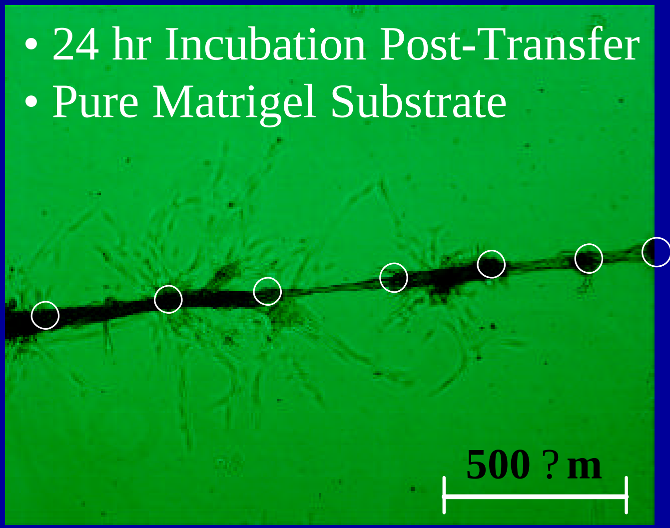
Single Shot; After 4 Days Culture Spread Over 700 Microns; 200 Micron Original Spot Size

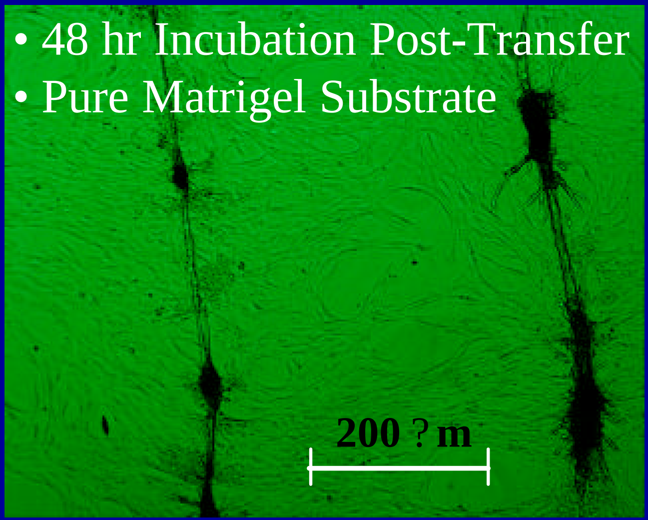


3 Shots; After 4 Days of Culture; Cells Did Not Spread; Bound in Cage/Matrix

Rat Cardiac Cells Behaved Differently Depending on the Local Environment!

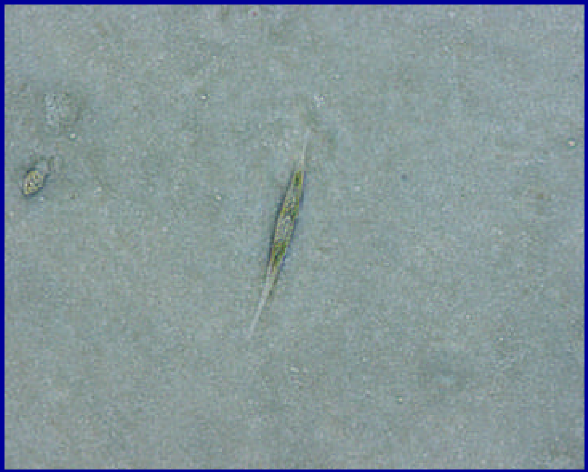
Forming Muscle-Like Structures Using MAPLE DW: Lines of Mouse Myoblasts





Mouse Myoblast Cell Spots Self-Formed Into 3-D Structures!

MAPLE DW of Human Dermal Fibroblasts

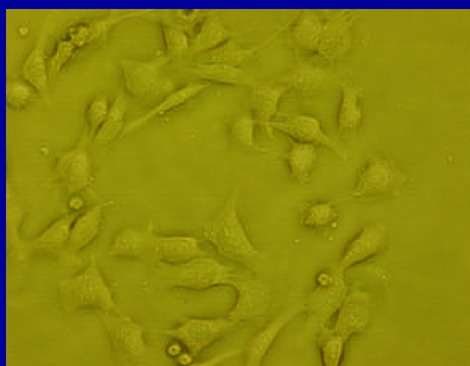


Single Cell

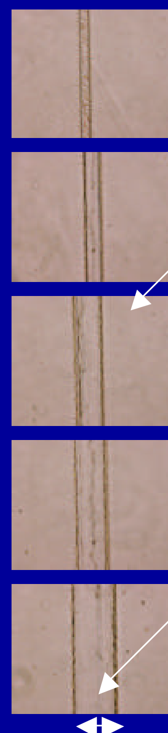
2-D Layer

Role of Laser Micro-Patterned Differentially Adherent Scaffolds in Aligning Myoblasts

Randomly Oriented C2C12 Muscle Cells In Culture

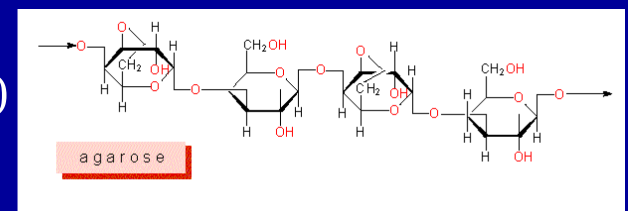


Myotube Channels



200 ?m

Agarose
(Non-Adherent)

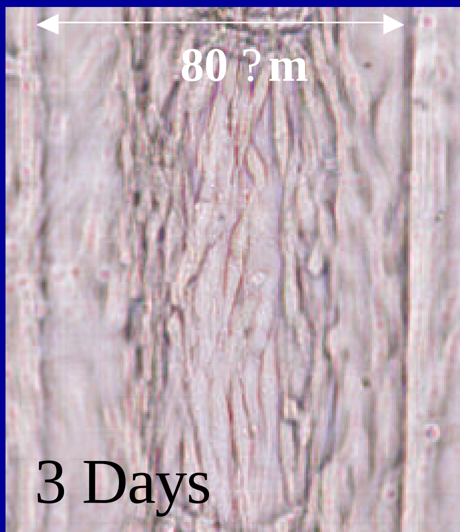


Matrigel Matrix
(Adherent)

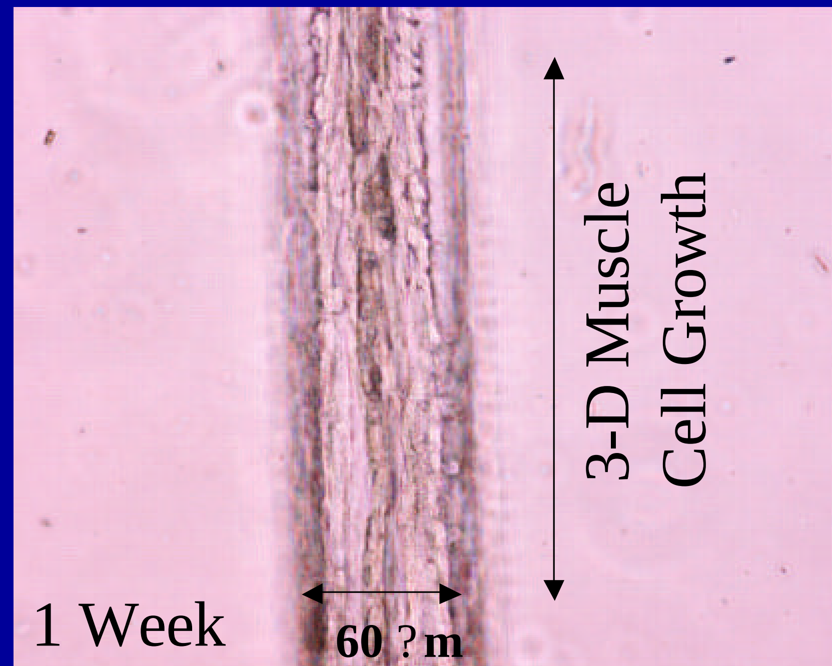
Solublized Basement Membrane Preparation:
(Laminin, Collagen IV, Heparan Sulfate Proteoglycans and Entactin)

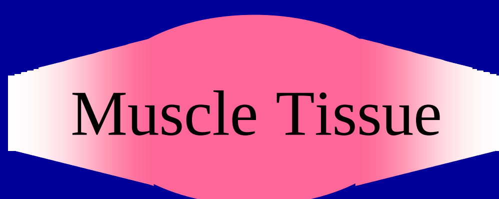
Controlled 2-D Adherence Forces Well-Defined 3-D Myoblast Growth?

CAD/CAM Alignment of 2-D Monolayers of Myoblasts to Control Myotube Orientation



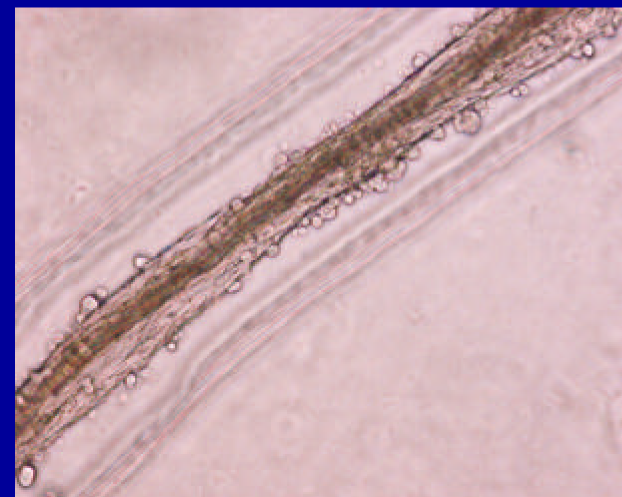
2-D Muscle Cell Growth





Control of Myotube Size and Formation By Differential Adherence and Matrigel Channel Width

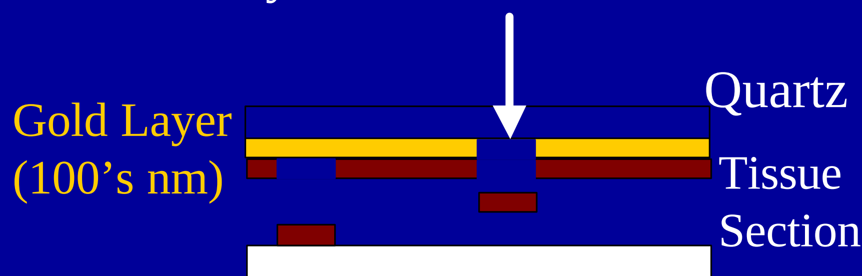
Circular Myotube Fabrication Using 2-D Differential Adherence



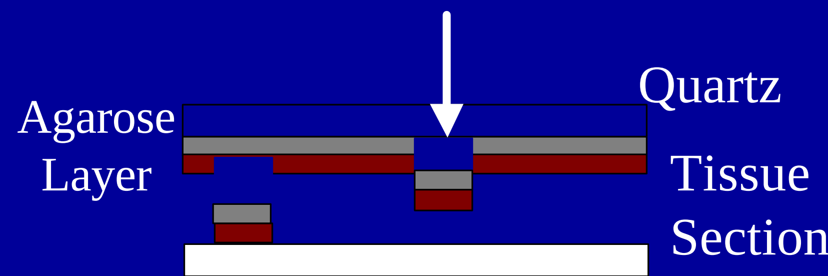
Controlled Fabrication of Circular Muscles!

Preliminary Tissue Section Transfers By MAPLE DW

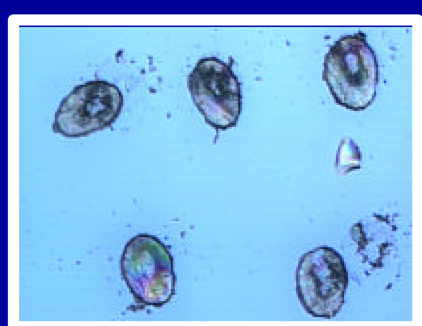
- Form Tissue Microarrays by Punching-Out 10 to 1000 ?m "Cookies" of Fixed Tissue (Live too?) and Depositing Them Onto Functional Glass Slides
  - Used Gold/Paraffin-Embedded Tissue Layered Structure
  - Used Agarose/Tissue and Agarose/Paraffin-Embedded Tissue Layered Structures



Gold Layer Is Sacrificial



Agarose Acts to Bind Tissue "Cookie" Together

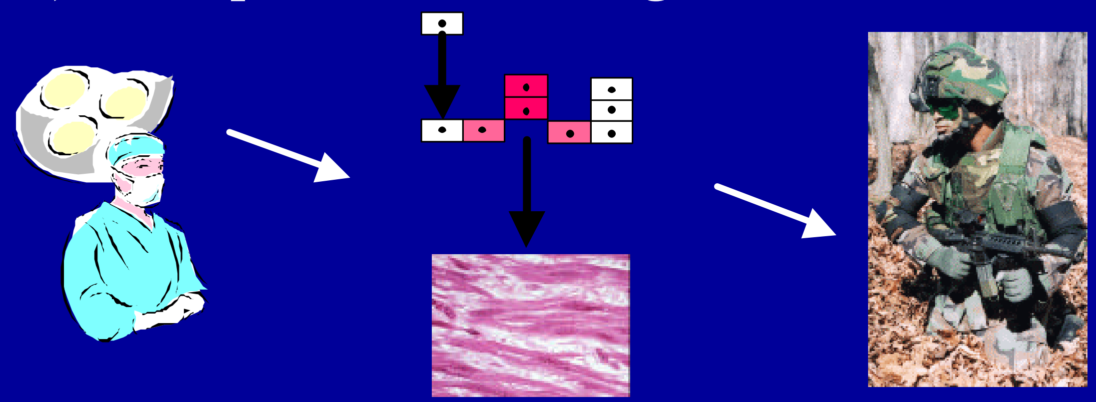


MAPLE DW Versus Other Biomaterial Transfer Techniques

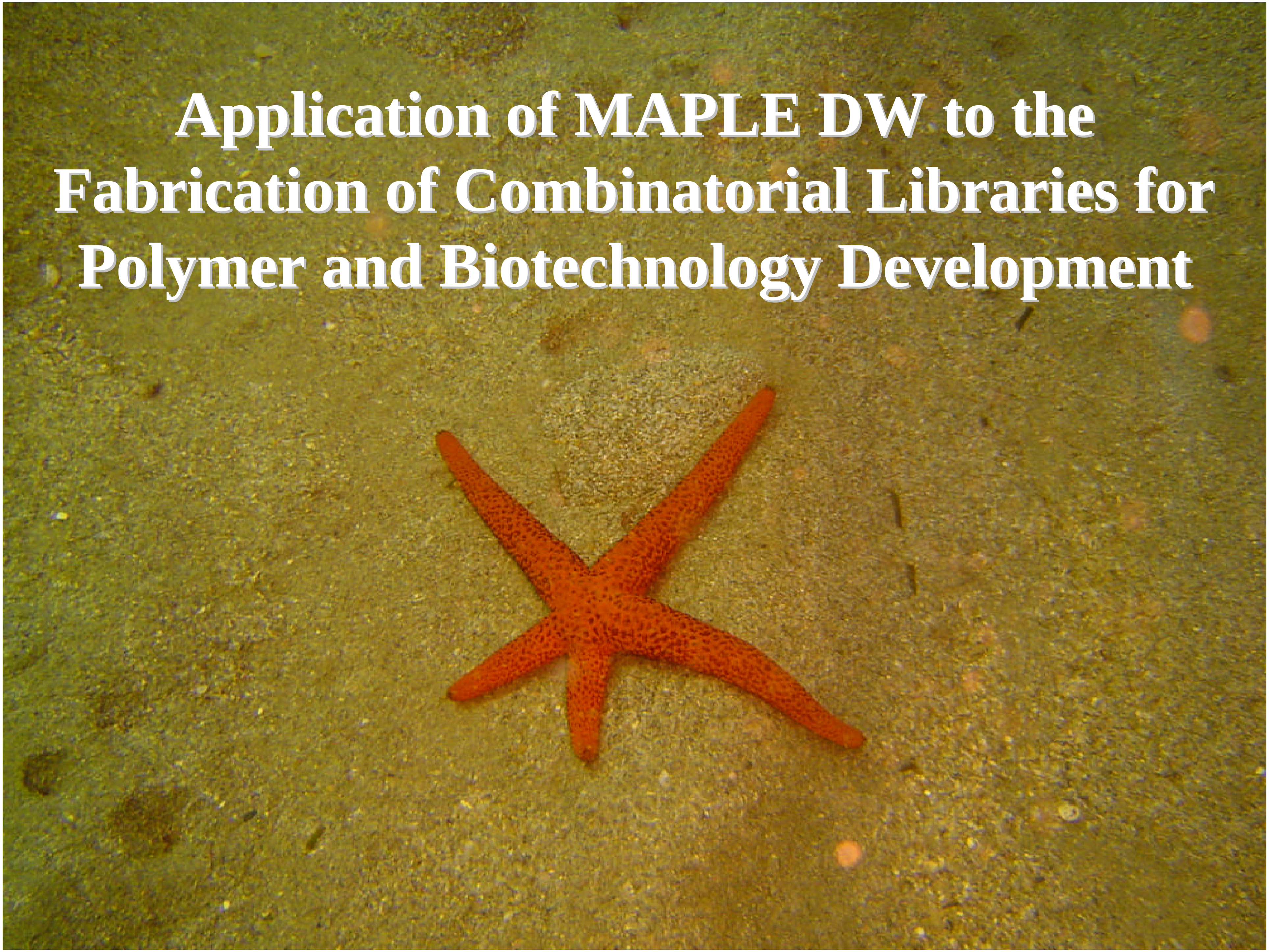
	Laser Capture Microdisection	Microcontact Printing (?CP)	Ink Jet	MAPLE DW
Capable of Patterning Living Cells on Substrates		●		●
Capable of Patterning Active Proteins on Substrates		●	●	●
Capable of Transferring Different Biomaterials Side-by-Side	●		● **with multiple dispensers*	●
Optical Imaging	●			●
Selectively Captures and Immobilizes Cells	●			●
CAD/CAM Compatible			●	●

Engineering Tissue-Based Structures and Devices Cell-By-Cell

- 3-D *Ex Vivo* Tissue Constructs (e.g., Hepatic, Immunological, Neural Living Systems)
- Tissue-Based Sensing of Warfare Agents and Environmental Toxins
- Cell Signaling Platform with Protein Identification Capability
- Cell Separation for Sub-Culturing by Living Microdissector
- Living Microfluidic Devices and Hybrid Biological Motors (Pumps, Valves, Dispenser for Microfluidic Chips)
- Battlefield Repair of Wounded Tissue (*In Situ* Tissue Re-Construction), Computer-Aided Surgeon



Application of MAPLE DW to the Fabrication of Combinatorial Libraries for Polymer and Biotechnology Development



Qualities of MAPLE DW for Combinatorial Processing

- Sub-Picoliter Voxels of Material
- CAD/CAM Process, 100 kHz Deposition Rate, Micron Resolution
- Capability to Work With All Polymers and Biomaterials
- Ability to Deposit Cells One-by-One
- Ability to Deposit 3-D Heterogeneous Structures
  - Engineered Tissue Constructs
  - Tissues Not Found in Nature
  - Living Microdissection
- .

CAD/CAM Tissue Culture Applications

Intracellular Activity:

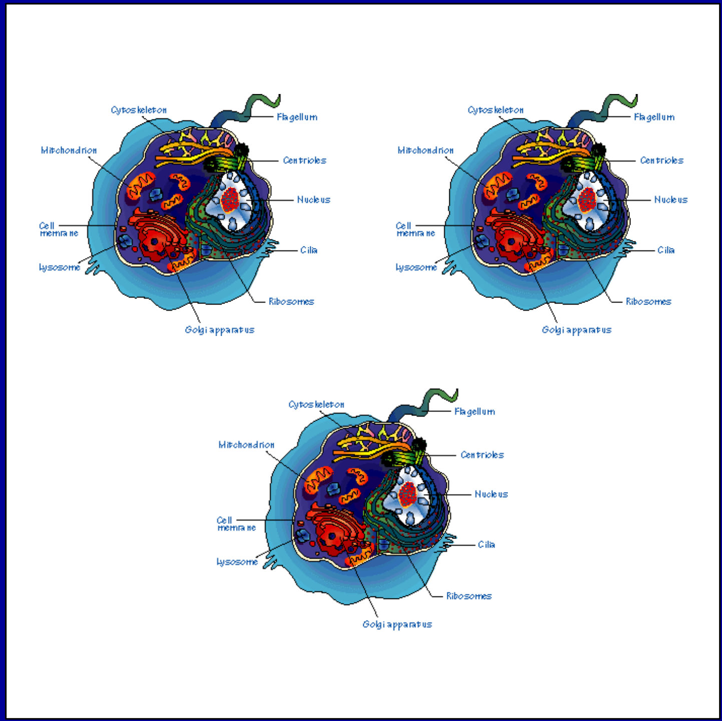
DNA transcription, protein synthesis, energy metabolism, drug metabolism, cell cycle differentiation, apoptosis

Intracellular Flux:

RNA, hormone receptors, metabolites, calcium, signal transduction, membrane trafficking

Environmental Int.:

Infection, drug action, ligand receptor interactions, cytotoxicity, mutagenesis, carcinogenesis



Cell Products:

Secretion, biotechnology, bioreactor design, product harvesting, downstream processing

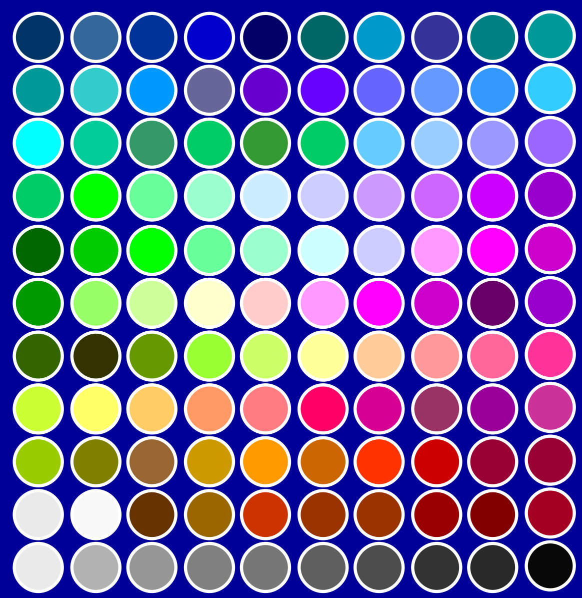
Genetics:

Genetic analysis, transfection, infection, transformation, immortalization

Cell-Cell Interactions:

Morphogenesis, paracrine control, cell proliferation kinetics, metabolic cooperation, cell adhesion and motility, matrix interaction, invasion

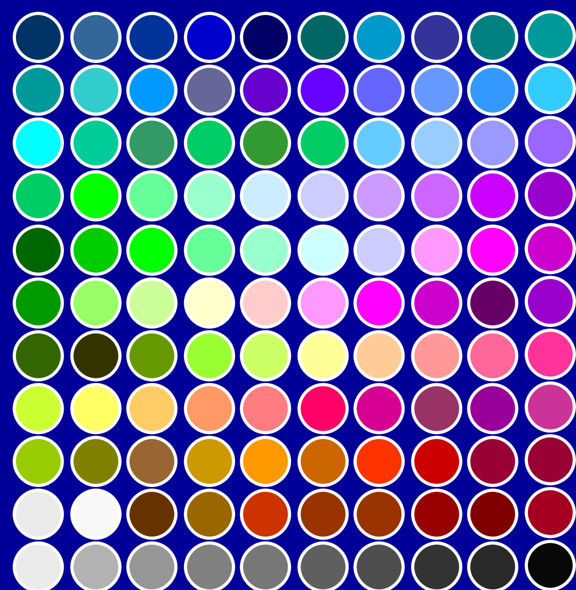
Fabricate a Library of Different Polymer Coatings to Test Dissolution Profiles for Controlled Drug Release



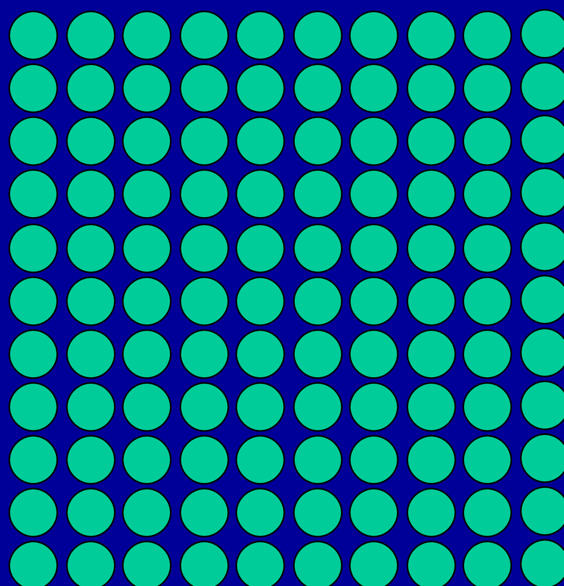
Polymer Library in Dissolution Environment

Apply a Specific Biomolecule Drug to a Library of Different Cells

Different Cell Structures

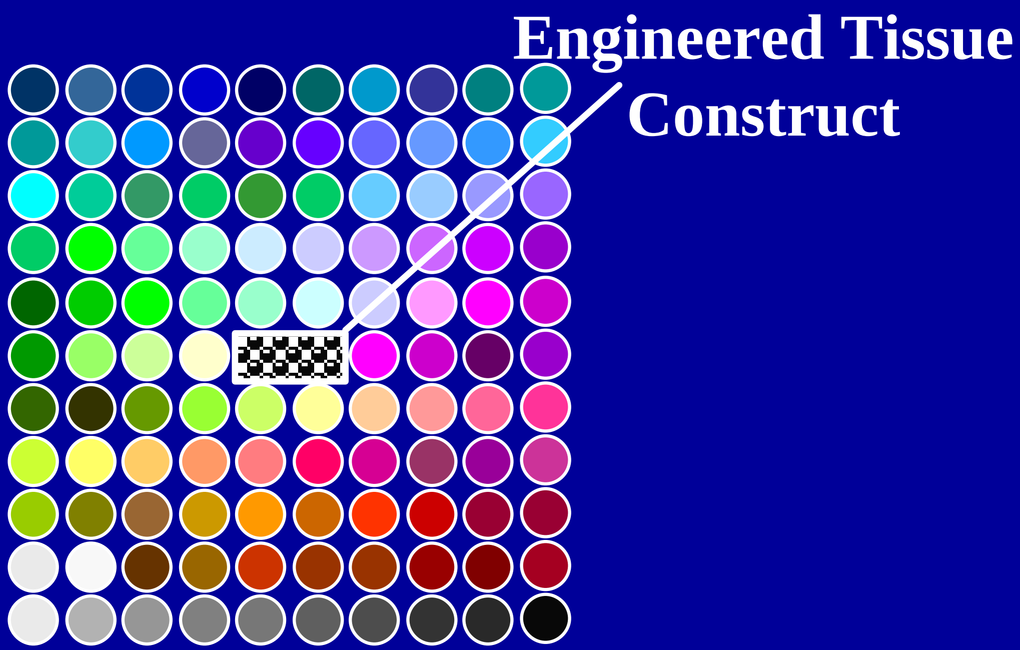


Apply a Library of Different Biomolecule Drugs to Any Number/Type of Cells



Single/Multiple Cells/Types

Engineered Tissue Construct Surrounded by Antibodies to Cell Signaling Proteins



Combinatorial Libraries by MAPLE DW: Conclusions

- New Era in Laser Processing of Polymers and Biomaterials.
- Parallel Investigation of Various Different Biomaterial/Biomolecule/Cell/Construct/Tissue Combinations.
- MAPLE DW Has Great Potential to Fabricate Unique Polymer and Biomaterial Combinatorial Libraries.